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JOURNAL OF MORPHOLOGY.

THE ORIGIN OF THE CEREBRAL CORTEX AND THE HOMOLOGIES OF THE OPTIC LOBE LAYERS IN THE LOWER VERTEBRATES.

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EDINGER's discovery of the cortical gray as distinct from the ventricular gray in the Reptilia (1, p. 111) leads naturally to the supposition that the rudimentary cortex, if nothing more, must be present in the amphibian cerebrum, in which the mantel is, in its relative size at least, not so very different from that of the reptiles.¹ And if we are able to find the homologous structure in Amphibia, what is the extent of that homology when we take into comparison the air-breathing vertebrates as a whole? The first part of this paper contains the result of my observations along that line. The second part consists of investigations directed to determining the homology, and in part the functions, of the several layers described by Osborn and others in the tectum opticum of *Rana* (2, p. 82) in comparison with those of the reptiles and birds.

The researches have been made under the supervision of Professor Osborn in the Class of '77, Biological Laboratory of Princeton College.

¹ This is not admitted by Edinger (1, p. 108). "Das erste was an diesen Schnitten auffällt, ist, dass keine Spur von einer Hirnrinde zu sehen ist."

PART I. THE CEREBRAL CORTEX.

1. AMPHIBIA. — In Amphibia the gray matter is mainly confined to the ventricular area, extending not more than one-third of the way out toward the surface (Fig. 1, *vg*). But at the inner corner of the cortex we find *a number of cells which must be considered as constituting the rudimentary cortical layer*. My first reason for taking this to be the rudiment, is the fact that the portion of the hemisphere where these cells are found coincides with the portion in the reptilian brain, where the differentiation of the layers is most complete; and the inference is, that the scattered cortical cells must be developed first in this portion before they make their appearance continuously in the mantle, in the form of a cortical layer. It is highly probable, therefore, that we shall find in the reptilian embryo a stage corresponding to the amphibian structure as just described. This statement is further justified by my observations in mammalian embryos, which, at a certain stage, present the reptilian structures as described subsequently; in short, by Von Baer's law, by which the higher forms repeat the structure of the lower in their ancestral history. The second and conclusive argument is, that these cells are situated superficially to the fibres of the corpus callosum, and consequently cannot be regarded as ventricular gray substance (Fig. 1, *cg*). In *Rana catesbiana* and *Menobranchus* these cells are irregularly arranged; but in *Spelerpes ruber*, from which the figure has been taken, a decided tendency to arrangement into a layer can be seen. Rows of from four to six cells are joined in lines parallel to the surface, making a sort of disconnected layer.

2. REPTILIA. — In *Tropidonotus* (Fig. 2) the cortical layer is distinctly developed, and in close examination it will be seen *that there are two cell-layers in the cortex*, specially marked at the inner corner. Thus making, in all, four layers for the cortex of *Tropidonotus*, viz.: first, the superficial white layer (Fig. 2, *f'*); second, the first gray layer (*cg'*) [Edinger's]; third, the second gray layer, or the layer of cells found among the fibres of the corpus callosum (*cg''*); and lastly, the ependyma (*ep*). This disposition of the two gray layers is especially interesting, because it corresponds to the typical structure of the cortical gray substance in Aves as well as in Reptilia, and thus appears to be the characteristic feature of the cerebrum of Sauropsida (Fig. 2, *cg'*, *cg''*).

There is in the *Tropidonotus* cortex a striking change in the appearance of the first gray layer, at the point midway between the inner and outer edges of the cortex. Outward from this point, the cells are considerably larger than those inside; they take a deeper carmine stain, and their cell-processes are very much more distinct. The change is rather abrupt. These cells continue downwards to the ventral outer corner of the hemisphere, and at this point terminate close to the surface.

Much the same structure obtains in *Emys* (Fig 3), excepting the last-mentioned characteristic, viz., the change in the size and appearance of the cells of the first cortical layer. There is also an indefinite fibre-layer between the first and the second cortical layers (*f''*).

3. AVES. — In *Columba livia* (Fig. 4) there are four layers corresponding approximately to those of the reptiles, but the cells composing the gray layers are very much more numerous than in reptiles; and, in fact, than in any other forms I have studied, not excepting *Didelphys*. First, there is a thin superficial fibre-layer; then comes the first gray layer, occupying not less than three-fifths of the entire thickness of the mantel. Some of the cells in this layer present a triangular appearance, but as far as I could ascertain, they are not of the pyramidal variety found in the mammalian cortex. The second gray layer is composed of cells running in the same direction as the fibres of the corpus callosum. Most of them are lenticular in shape, but there are a number that are less elongated.

4. MAMMALS. — A lower type of the class—the opossum—was selected in order the more easily to find the homology, should any exist, to the preceding forms. In the opossum there are seven layers altogether: I, superficial fibre-layer; II to V, four gray layers; VI, fibre-layer of the corona radiata; VII, the ependyma (Fig. V).

The first gray layer (or the layer II of the figure) consists of numerous pyramidal cells among which are also seen some of the ordinary spherical cells. The pyramidal cells have each a nucleolus, and their apices are turned toward the surface. The layer III is composed exclusively of ordinary nerve-cells, and their processes present no definite trend. In this layer vertical fibres are visible, as is also the case with the layer II. The layer IV resembles the II in its composition; only the pyramidal cells

are slightly larger than those of the II, and they are very much less in number. Next is the layer of ordinary cells, through which pass the fibres of the corpus callosum.

It seems very likely that the first three (II–IV) of the four gray layers have been developed from the first cortical layer of the Sauropsida (cg'); in other words, I am led to homologize these with each other, and also the layer V with the second cortical layer (cg''). I find in the young squirrel embryo (*Sciurus*) a stage corresponding to the sauropsidan structure; the first gray layer is very thick, and the second is not so rich in cells as the first. I was not fortunate enough to obtain a stage in which the first gray would be in the process of differentiation into three, but the general disposition was such as to justify, in my opinion at least, the conjecture stated above.

To summarize: we find in the cells of the gray matter surrounding the ventricle a tendency to migrate toward the superficies. This process, as we have seen, is in progress in the Amphibia, and initiates the reduction of the ventricular gray, from its condition as the main cellular element of the hemispheres to its entire absence in the adult condition of the higher forms.

PART II. OPTIC LOBES.

I. AMPHIBIA. — The mesencephalon in the Urodela is a tubular structure, — gray substance lining the central cavity, the mesocœle, and showing, in most cases, no differentiation. In some salamanders (*Spelerpes*), however, the cells are arranged in concentric rows, and at places these rows, leaving little spaces between them, anticipate the more complex structure of the Anura. In the latter the mesencephalon assumes a bilobular structure and forms a prominent portion of the brain. Professor Osborn (2, p. 82), in his "Internal Structure of the Amphibian Brain," has described eight distinct layers in the tectum opticum of *Rana*, — four white layers alternating with the four gray layers, and also traced the optic nerve-tract into the first and second white layers.

These layers in *Rana* are so beautifully differentiated that they may be taken as a standard of comparison for those of the Sauropsida. Taking them in order from without inwards, the characters of these layers are as follows: The layer [*A*] (Fig. 6)

consists chiefly of fibres which are traceable to the optic nerve. Some of these fibres sweep over the tectum opticum and pass into the Thalamencephalon (and ultimately into the cerebrum). [B.] This is the first gray layer, consisting of loosely arranged cells. These cells are of the spherical description, and the majority of the polar processes point toward the mesocœle. [C.] The second white layer differs from the first in two respects: it contains vertical fibres descending from the layer B, and secondly, none of its fibres seem to pass toward the hemispheres. The layer [D] is a compact cell-layer. These cells are somewhat spindle-shaped, the processes running radially. They are arranged in from seven to nine concentric rows, and at places one or more of these rows are seen separated from the rest. Anteriorly, in front of the mesocœle, this layer forms a wide band owing to the interspaces of fibres formed between each cell-row, and along the proximal side of the lobe it is pushed apart to admit the exit of the Trigeminal nerve-tract, which proceeds from the layer F, as will be described further on (Fig. 10), and also for the fibres of the *commissura tecti optici*. [E.] The fibres descending from the layer D to F, the commissural fibres apparently connecting the layers D and F of the opposite sides, and probably fibres coming from the cerebrum constitute the afferent portion of the third white layer (Fig. 6, E) (Fig. 10). The efferent portion is composed of fibre-tracts which, according to my observation, partly contribute to the III, IV, and V (probably the VI also) nerves, starting from the layer F. The tract contributing to the third nerve arises from the ventral portion (below the mesocœle), that of the fifth nerve from the proximal side, and those of the fourth and sixth from the dorso-posterior portion. The tract of the VI probably passes through the "nucleus magnus"; but my observation as to the course of this tract has been very incomplete. [F.] The layer F is marked by the presence of large multipolar cells, constituting the well-known mesencephalic nucleus of the Trigemini. They each have a nucleolus, and are situated mainly in the anterior and proximal portions, where the fibres of the III and V nerve-tracts, respectively, are very distinct (Figs. 10 and 11).² The greater part of the cells composing this layer are rather spindle-

² The multipolar cells are drawn rather too large in these figures. The real proportion is represented in Fig. 6.

shaped and proximal to the mesocœle; are in single row pressed side by side. Posteriorly the cells are more numerous, and in the anterior and ventral portions they expand to form a wide band, as has been mentioned in reference to the layer *D*.

[*G.*] In this layer fibres or striations are seen connecting the ependyma below with the layer *F* above. There are also some fibres running around the ependyma layer (*H*, Fig. 6).

2. HOMOLOGIES IN THE CORPORA BIGEMINA OF THE SAUROPSIDA. — Although in reptiles and birds the structure of the optic lobes is not so clearly marked as it is in the frog, yet the homology of the layers in these forms is not difficult to trace. Beginning with the layer *A*, we find it constant in *Tropidonotus*, *Emys*, and *Columba* (Figs. 7, 8, 9, 10, *A*). These fibre-layers are traceable to the optic nerve, as is the case in *Rana*. They are, however, much reduced in thickness, especially in *Columba*. In *Tropidonotus* (and in most places in *Columba* also), this layer is not well differentiated from the layer *B*, which, in turn, is not marked off from the layers below (Fig. 7). In the turtle it is slightly better marked; but, owing to the presence of cells in the next layer, it is not so clearly brought out as in *Rana*. In *Columba*, layer *B* is distinct at the inner corner of the tectum opticum from which Fig. 9 has been taken. Layers *B*, *C*, and *D* are not differentiated in *Tropidonotus*, while the latter two (*C* and *D*) are not distinguishable in the turtle and pigeon. The layer *CD* consists of fibres and cells, representing the two distinct layers in the frog. The cells are less spindle-shaped than in the frog, although the fact that their processes run mainly upwards and downwards (or strictly speaking, radially) makes them appear rather elongated in the same direction. In *Tropidonotus* cells are irregularly scattered about, but in *Emys* they are more numerous toward the mesocœle; the rows of cells bordering the layer *E* being somewhat larger than the rest (Fig. 8, *CD*; Fig. 7, *BCD*). *Columba* exhibits a high degree of differentiation toward the inner edge of the tectum opticum. This layer is divided up into three sublayers of cells, with fibre-laminæ intervening between them (Fig. 9, *CD*). The character of the cells is similar to what is found in preceding forms.

E, or the layer of the commissura tecti optici, is conspicuous in all the specimens. It is thicker in *Tropidonotus* than in either *Emys* or *Rana*. *Tropidonotus* also presents a singular

arrangement of *E*. This fibre-layer in its lower half is crowded by cells which apparently have extended outward from the layer below. These intruding cells are lenticular in shape, with their longest diameter arranged radially (or vertically in Fig. 7). In *Columba* it is subdivided into two strata, — an outer stratum of horizontal fibres (of the commissure), and an inner stratum of vertical fibres. These strata are further distinguished by the presence of cells different in each. But as I am inclined to consider these cells as properly belonging to the layer *F*, they will be described later on.

In *Tropidonotus* the layer *F* is well provided with large multipolar cells, with nucleoli (*mesencephalic nucleus of the Trigemini*), and the ordinary cells are much more abundant than in *Rana*, but are not packed side by side, as we have seen them in the latter. Besides, there are, as has been mentioned, in connection with *E*, a number of lenticular cells proceeding upward from *F* (Fig. 7, *F*). Although in *Emys* the large cells are not nearly so numerous as in *Tropidonotus* (being mainly confined along the junction of the lobes), their scarcity is compensated, as it were, by the elaborate stratification observed in this layer. In most places there are five strata divided by fibre-laminæ. These strata are composed of ordinary cells which are somewhat spindle-shaped. Returning now to those cells found in the layer *E* of *Columba*, which form the first two sublayers of *F*, we observe, first, in the horizontal stratum, or among the fibres of the Commissura tecti optici, that the large multipolar ganglion cells are elongated in the direction of the fibre-tract; but the smaller cells do not present any definite direction. Secondly, in the vertical fibre-stratum, ganglion cells do not present any definite direction, while on the other hand the lenticular cells run vertically; that is, in the trend of fibres. The third stratum, or the layer *F* proper, consists of ganglion and small spherical cells with nucleoli and with processes extending in all directions.

The layer *G* has no marked feature, except in *Columba*, where it presents more of the appearance of molecular structure than distinctly fibrillar. The ependyma is thickest in *Rana*, consisting of from five to six rows, while in *Tropidonotus* and *Columba* it is very thin (Figs. 6, 7, 8, 9, *H*).

3. FUNCTIONAL RELATION OF THE OPTIC LOBE LAYERS.

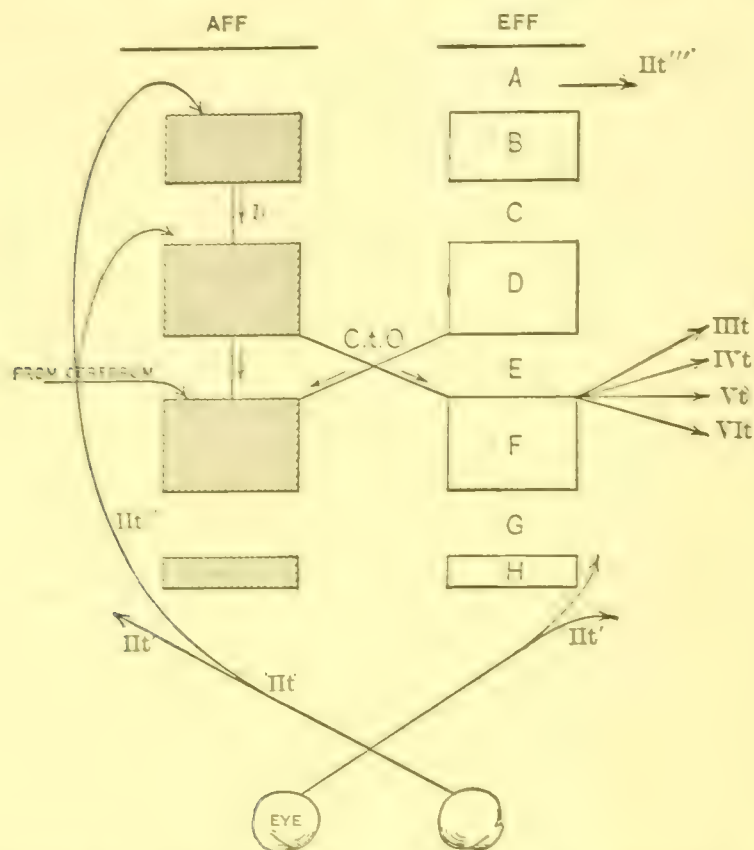


Diagram showing the supposed relations of the layers of the optic lobes. Each lobe is composed of afferent and efferent portions; but the figure represents (for the sake of clearness) the afferent portion of the one and the efferent portion of the other side.

The above diagram is intended to represent the conclusions I have reached by the preceding investigations.

The optic nerves decussating at the chiasma sweep around and enter the lobes in the opposite sides, at the distal and posterior portions (*III't''*). These tracts can be traced to layers *A* and *C*, as has been described above. Many of the fibres (*III't''*) of the layer *A* pass over the tectum opticum and enter the thalami, and thence probably into the cerebrum.³ As there are descending fibres (*b*) from the layer *B*, it is natural to infer that they are those of the optic tract of the layer *A* turned out of their course by the agency of cells constituting the layer *B*. Most, if not all, of the fibres of *C* are turned from their course by the

³ I have observed in *Tropidonotus* the tract going direct (without passing through the tectum opticum) to the thalami (Fig. 12, *III't'*). In other forms my observation has not been complete.

layer *D*, and a great portion is reflected so as to enter the cell-layer *F* of the opposite lobe. The reflected fibres from the opposite sides together constitute the *commissura tecti optica*. So far, the nerve-impulse through the fibres has been sensory. In layer *F* the conversion into, or relation with, motor impulses must take place, because we find fibres and cells from this layer supplying directly the oculo-motor and trigeminal nerves, and indirectly, probably, through the *nucleus magnus*, the trochlear and abducens. The presence, constant in all the forms, of the large ganglion cells of the mesencephalic trigeminal nucleus confirms this hypothesis. In the layer *F*, therefore, we are to look for reflexes of the III, IV, V, and VI nerves (Fig. 12, diagram). And consequently the eye-muscles and other parts supplied by those nerves may be excited into activity in a reflex manner. Furthermore through the relations these tracts have with the centres in the Medulla, this may serve to explain the co-ordinated reflex movements exhibited by animals in which the cerebrum has been removed. I have also observed fibres which appear to show that the tracts coming from the cerebrum enter into the layer *F*. In this case the cerebral voluntary impulses would also run through this tract. This point, however, must be confirmed by further observation.

This is of course a tentative hypothesis of the complex relations of the optic lobe layers to each other, to the cerebrum and to the origin of the cranial nerves; it is, nevertheless, along a line of observation which has not to my knowledge been attempted before, and which it is evident will lead to definite results.

PRINCETON, August, 1889.

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EXPLANATION OF PLATE.

FIG. 1. Cerebrum of <i>Spelerpes ruber</i> . Transverse section.	× 40.
FIG. 2. The same of <i>Tropidonotus</i> .	× 18.
FIG. 3. The same of <i>Emys</i> .	× 20.
FIG. 4. Of <i>Columba livia</i> .	× 80.
FIG. 5. Of <i>Didelphys</i> .	× 120.
FIG. 6. Part of the transverse section of the tectum opticum of <i>Rana</i> .	× 110.
FIG. 7. The same of <i>Tropidonotus</i> .	× 180.
FIG. 8. Of <i>Emys</i> .	× 150.
FIG. 9. Of <i>Columba livia</i> .	× 90.
FIG. 10. Transverse section of the optic lobes of <i>Rana</i> .	× 18.
FIG. 11. Vertical section of the same.	× 20.
FIG. 12. Vertical section of the optic lobe of <i>Tropidonotus</i> .	× 22.

A, 1st white layer of the tectum opticum. *B*, 1st gray layer. *C*, 2d white layer. *D*, 2d gray layer. *E*, 3d white layer. *F*, 3d gray layer. *G*, 4th white layer. *H*, 4th gray layer. *BCD*, layer corresponding to the layers *B*, *C*, and *D*, in *Rana*. *CD*, layer corresponding to the layers *C* and *D* in *Rana*.

III, *III'*, *III''*, *III'''*, tracts of the optic nerve. *III**t*, tract of the oculo-motor nerve. *IV**t*, *V**t*, *VI**t*, tracts of IV, V, and VI nerves respectively. *Vn*, nucleus of the Trigemini. *b*, fibres descending from *B* to *D*. *cal*, corpus callosum. *cg*, cortical gray. *cg'*, 1st cortical gray layer. *cg''*, 2d cortical gray layer. *c. t. o.*, commissura tecti optici. *Di*, diencephalon, or the optic thalamus. *Ep*, ependyma. *f'*, superficial white layer. *f''*, 2d white layer. *F. b. b.*, basal prosencephalic tract. *For*, fornix. *msc*, mesocœle. *Mt*, metencephalon. *prc*, procœle. *pres*, anterior commissure. *vg*, ventricular gray.



THE SKELETAL ANATOMY OF AMPHIUMA DURING ITS EARLIER STAGES.

By O. P. HAY.

IN the *American Naturalist*, Vol. XXII (1888), p. 315, the writer has published an account of the finding of the eggs of *Amphiura*, and accompanied it with a short description of the anatomy of the embryos contained in those eggs. In the present paper it is proposed to enter somewhat more into details in describing the structure of the skeleton of the young *Amphiura* and to illustrate the descriptions by drawings.

As stated in the communication referred to, these eggs were found in a cypress swamp at Little Rock, Ark., on Sept. 1, 1887. They had been deposited in a small excavation under an old log, which was lying at a distance of some rods from the nearest water; and were being cared for by the mother, who was lying coiled up around them. The mass of eggs was about as large as one's fist, and, being connected in strings, they greatly resembled a mass of large beads. When the eggs were put into alcohol, the young were seen to move about within the eggs.

The egg-strings were so entangled that it was found to be impossible to separate them, for the purpose of determining the number of strings and of eggs. However, since there were four ends visible, it is supposed that there were two strings, one for each oviduct. The number of eggs is estimated to be at least one hundred and fifty. They are globular in form, and have an average diameter of 9 mm. (See Fig. 1.) They are separated by from 5 to 12 mm. of string. Fourteen eggs were counted on a piece of string 225 mm. in length. Each egg, in the condition in which they were discovered, consists of the contained larva and an external capsule. This latter is of a condensed gelatinous material, thin as paper, becoming brittle in strong alcohol, but swelling somewhat in weaker alcohol and in water. The connecting cords are of the same materials, and have a diameter

of from 1.5 mm. to one-half that size. The capsules are almost entirely filled up by the young amphiumes; but in their fresh state they probably contained also some water. The young are coiled within the eggs in various positions, so as to form about three turns of a spiral. On being taken from the eggs and extended, they are about 45 mm. in length (Fig. 2). So far as I have been able to discover, they are all at the same stage of development. The color above is dusky, with a slightly darker dorsal streak and a similar lateral band. Below, the color is pale. In the alcoholic specimens the belly appears yellow on account of the great amount of yolk that is contained within it.

In form and proportions the young amphiume is stouter than the adult, and the head is broader and more depressed, and the snout more rounded. The head, therefore, resembles more nearly that of the typical Urodeles than does that of the adult. The eyes also are more conspicuous than are those of the more mature animals, and would doubtless, for some time after hatching, be of more service. The fore and the hinder limbs are present, but are diminutive in size. On the anterior limb, three toes are indicated; the hinder limbs give little evidence of separation into digits. The tail differs from that of the adult, inasmuch as it has on both its upper and lower edges a distinct membranous fin.

The larvæ possess conspicuous gills; and since they are evidently near the period of hatching, it becomes quite probable that these gills will be retained for some time after the young have betaken themselves to the water, their native element. The gills consist of three pairs, and are of the simply pinnate form. The second gill is the longest, measuring about 9 mm. in length, and gives off from the main stem ten delicate twigs. Only once have I observed any of these lateral filaments to divide. The first and third gills are somewhat shorter, and have about eight lateral branches each. In all the main stems and the lateral twigs may be seen arteries and veins filled with the coagulated blood. Three gill-slits are still open, the first and second of which become closed in the adult.

We cannot but be struck by the close resemblance that exists between the breeding habits of the *Amphiuma* and those of *Epicrium glutinosum* of Ceylon, as these are presented to us by Messrs. P. B. and C. F. Sarasin in the "*Arbeiten aus dem*

Zoologisch-zootomischen Institut in Würzburg," Bd. VII, 1885. According to these observers, the female of *Epicrium* excavates a cavity in the earth a little below the surface, and there deposits a mass of eggs, which are connected by means of an albuminous cord, and thus resemble a string of pearls. These eggs, when found in the oviduct, are of an oval form, about 9 mm. through the longer axis and 6 mm. through the shorter; but, when found in the earth, they were about twice as large, having probably during their development absorbed considerable water. Unlike *Amphiura*, the eggs seem to have something of a regular arrangement in the mass, the connecting strings being bent in toward the centre of the mass and cohering there into a viscous knot. Around this mass of eggs the female lies coiled, and gives them protection. The embryos at the stage described were 4 cm. long, and moved actively about. On each side of the neck were three plume-like gills, the longest of which was about 2 cm. The eyes were relatively large and distinct; while the tail was surrounded by a strongly developed fin-membrane. These eel-like young must greatly resemble those of *Amphiura*. Other larvæ of *Epicrium* were found by the Messrs. Sarasin swimming in the neighboring brooks. These are without gills, possessed yet gill-clefts and a caudal fin, and are said to attain a length of perhaps 16 cm. At length their transformation is completed, and they leave the water. These facts bearing on the mode of embryonic development and others derived from anatomical structure have made it evident to the writers named above, as well as to others, that the Cœcilians must be arranged very closely to the Urodela, if not consigned to the same order. The habits of oviposition and incubation and the course of development which have now come to light as characterizing *Amphiura*, must tend to strengthen this opinion.*

It is not yet known how the young amphiumes get to the water after they have been excluded from the eggs. Considering the nature of the ground where the eggs were found, it would appear impossible for them to travel any considerable distance. It might well be, however, that, like many other

* Dr. C. O. Whitman kindly sends me the interesting information that the Giant Salamander of Japan (*Megalobatrachus maximus*) lays its eggs in a string exactly like that of *Amphiura*, and the eggs are of about the same size.

Amphibia, their development would be retarded until some fortunate day when a heavy rain would make it possible for them to reach permanent water.

I now proceed to describe the structure of the larval *Amphiuma* as disclosed by the contents of the eggs under consideration. In my attempts to unravel this structure, I have depended partly on dissections made by means of lens and needle, but mostly on stained sections cut and mounted serially.

I. THE SKULL.

In Fig. 3 we have a view of the cartilaginous cranium seen from above, and in Fig. 4 a view of the same from the side. What will probably first strike the attention of the observer is the existence, in the basilar region, of two fontanelles in the cartilage, one on each side of the middle line. They are of an elongated oval form, and are of such size that they leave only a narrow strip of cartilage between them, and a similar ledge along the inner and lower border of each otic capsule. Both before and behind these fontanelles, the cartilage passes from one side of the skull to the other. This portion of the primitive skull is worthy of comparison with the adult skulls of *Necturus* and *Siren*. In *Necturus* the trabeculæ cranii are not connected by cartilage behind the pituitary region, and have but a narrow band connecting them in the exoccipital region; so that there is no cartilage in the floor of the brain-case behind the ethmoidal region until immediately in front of the foramen magnum. In *Siren* there exists, opposite the middle of the otic region, a band of cartilage that passes from side to side. Behind this, there is in the middle line a single fontanelle; and this is limited behind by the cartilage of the basioccipital region. The anterior ends of the two fontanelles of *Amphiuma* come much further forward than does the single one of *Siren*.

In the narrow strip of cartilage between the two basal fontanelles is seen the anterior end of the notochord extending well forward toward the pituitary space.

In the exoccipital region the condyles project prominently backward, after the manner of those of the adult. The deep notch between them is occupied by the tooth-like process of the

atlas. On each side, at the base of the condyles, are the foramina for the vagus and the glossopharyngeal nerves. The occipital condyles are invested with a thin ectostosis, which continues as far forward as the foramina mentioned. This is the only cartilage-bone that is found in the skull, except that in the hyobranchial apparatus, soon to be described.

In the supraoccipital region, there is a narrow strip of cartilage which arises from the posterior end of the otic capsule, and extends inward toward the middle line, but it lacks much of reaching the corresponding cartilage of the other side. Along its upper surface, therefore, the brain, from one end to the other, has no other protection than that of the integument.

The otic capsules are large, well-developed, and of a long-ovoidal form. They occupy about one-third of the total length of the cartilaginous skull. The upper surface is somewhat flattened, and slopes outward and downward. Anteriorly they pass by a narrow band of cartilage into the upper edge of the trabeculæ in front of the foramina for the fifth pair of nerves. Behind, each capsule is rounded, and in the angle between it and the projecting condyle, is found the vagus ganglion. The membraneous canals are well-developed, and may be seen through the walls of the capsule. They are enclosed within corresponding cartilaginous canals. On the outer wall of the capsule is found the large fenestra ovalis. It is partially occupied by the cartilaginous stapes, as shown in Fig. 4. All round this stapes is a tract of membrane, except anteriorly, where it is articulated to the otic wall. This stapedia cartilage is confluent with the hinder end of the columella, which will come up for consideration in its place. With the exception of the fenestra ovalis, there is no interruption in the cartilage of the outer wall of the otic capsule. The facial foramen lies immediately in front of the fenestra ovalis. At the anterior end of the capsule, and on the lower floor, is found the entrance of one portion of the auditory nerve into the labyrinth. Farther back, about opposite the fenestra ovalis, there are three openings in the cartilage of the mesial wall of the otic capsule. The smaller one, high up, is for the passage of the ductus endolymphaticus into the brain-cavity. A second larger foramen in the cartilage, immediately below the last mentioned, admits into the labyrinth the branch of the auditory nerve going to the sacculus and lagena. This

nerve will receive further attention. Immediately behind the foramen just considered is a third break in the cartilage, the purpose of which I have not been able to determine. It possibly gives passage to some of the lymph sinuses.

In sections made previously to decalcification, there are seen abundant otolithic deposits.

In front of the otic capsules, the trabecular walls are low and slope gently downward and inward. The foramen for the branches of the trigeminal nerve is large, and is traversed by the long, slender, ascending process of the suspensorium (Figs. 3 and 4, *As.p.*), which process passes between the orbito-nasal and the other branches of the fifth nerve. Anteriorly to this foramen the cranial walls, becoming lower, approach each other gradually until they finally meet and coalesce, and thus enclose the ovate-acuminate pituitary space. A little further forward, the trabeculæ again separate into the cornua.

In the low trabecular wall, just behind the eye, are found two foramina. Through the most anterior passes the optic nerve. The posterior possibly admits the passage of the oculo-motor; but this I have not been able to demonstrate.

In front of the optic foramen, there is given off from the upper border of the trabecular wall a rod of cartilage which extends outward and forward to a point just in front of the eye, and above the hinder end of the nasal-sac. Here it expands into a rudimentary capsule for this organ (Figs. 3 and 4, *Na.C.'*).

From the point where the above-mentioned rod leaves the cranial wall, the trabeculæ are slender and rod-like, but increase somewhat in size to their coalescence in the ethmoidal region. The lateral halves of the ethmoidal cartilage slope downward and outward. There is no trace of a naso-septal lamina. The trabecular cornua are bilobate, one portion of each (Figs. 3 and 4, *C.t.'*), forming a plate that curves outward under the nasal-sac; the other running forward, at first a little outward, then downward and inward, until it terminates in a point close to the base of the ascending process of the premaxilla (Figs. 3 and 4, *C.t.*).

Just below the eye there is a short piece of cartilage that stands outward and forward from the trabeculæ, to which it is joined by means of connective tissue. This is the antorbital (Fig. 4, *Ant.*). Running parallel with the trabecula along its

outer border, and between the antorbital cartilage and the trigeminal foramen, is a slender club-shaped tract of cartilage, whose position is shown in Fig. 4, *Pl.* It appears to represent the pterygoid cartilage of other Urodeles; but it has not yet formed a connection with the suspensorium. It lies about its own diameter outside of the trabecula.

The eye is wholly devoid of any cartilaginous capsule.

Another cartilage that I have sought for with great interest is that which appears in the roof of the mouth of the adult, in front of and between the anterior ends of the vomers and below the palatine process of the premaxilla. Not a trace of this cartilage is seen in any sections that I have examined.

The floor of the brain-case just described is concave from side to side. Proceeding forward from the foramen magnum, the floor, as shown in a longitudinal section along the middle line, slopes rapidly down beneath the hind-brain, then horizontally forward to the middle of the cerebrum, where the slope is again upward.

Coming to the post-oral structures, we observe first that Meckel's cartilage comes forward and meets its platetrope, while posteriorly it projects behind the articulation with the suspensorium, and gives insertion to the digastric muscle. This cartilage is ensheathed by membrane-bones, which will be considered further along; but it shows no signs of a deposit of calcific matter to form the articular.

The suspensorium (Fig. 4) is of a quadrate form, is directed slightly forward, and in transverse sections is broader below than above. It articulates with the auditory mass by means of the otic process and the pedicle. Starting from the lower end and inner border of the suspensorium is the long and slender ascending process, which runs upward and forward, and coalesces with the cranial wall at the anterior side of the trigeminal foramen. I find no trace of a pterygoid process. From the hinder border of the suspensorium (Fig. 4) starts out a short process which articulates with the columella auris. No ossification has as yet appeared in the suspensorium.

The columella is a short rod of cartilage, which, articulating with the suspensorium anteriorly, runs backward and coalesces with the outer surface of the stapes. Its relation to the facial nerve will be discussed later.

The hyobranchial apparatus (Figs. 4 and 5) consists of the hyoid arch and four branchial arches. The hyoid arch presents, on each side, a hypohyal and a ceratohyal. The basihyal has not as yet become chondrified. There is a single basi-branchial as in the adult. The four branchial arches are much as in the mature animal. There is no second ceratobranchial present. Huxley states that there is one present in the adult, but it is not represented in Wiedersheim's figure. The ossification connected with the hyobranchial arches will be referred to immediately.

Ossifications. — It has been already stated that the exoccipitals are undergoing ossification. These ectostoses do not meet in the lower middle line in front of the foramen magnum.

The only other ossification of this kind occurs in the first branchial arch. A delicate but easily distinguishable layer of bone invests the slender portion of the lower end of the cartilaginous bar, as shown in Fig. 5.

The following parostoses occur in this skull: premaxillary, vomers, parasphenoid, frontals, parietals, squamosals, dentaries, angulars, and a hyoidean splint. In my determination of the presence and the relations of these bones, as well as of the two cartilage bones, I have carefully compared them with the ossifications found in the skulls of larval *Amblystomas*. I have also, in the case of nearly all of them, been able to dissect them out, clean them, and apply chemical tests.

The premaxillary of the adult is a very remarkable bone; it is no less so in the case of the embryo. In the adult the lateral halves are so completely consolidated that no evidence is afforded by them that they ever have been distinct. It is composed of two alveolar processes: an ascending process, which runs backward between the nasals and the frontals to a point a little behind the line joining the anterior borders of the orbits; and a palatine process, which appears in the roof of the mouth between the vomers nearly as far back as their hinder ends and underlying the parasphenoid. It appears to be this process which has been described by several authors as a sphenoidal ossification. For nearly half their length anteriorly these two processes are connected by a thin plate of bone which functions as a nasal septum. Nearly the whole remaining space between them is occupied by cartilage. Dr. Wiedersheim

(*Kopfskelet der Urodelen*) seems to have been the first to describe correctly this structure, especially the relation of the palatine process to the premaxilla. He endeavors to explain this remarkable bone by suggesting that we have in it a composite of morphologically different elements. In the ascending and the alveolar portions of the bone there is supposed to be the proper premaxillary. In the osseous nasal septum and palatine process we have an ectosteal (*perichondrostotisch*) bone formed from the originally hyaline nasal septum, which bone has become confluent with the proper premaxillary. The fact that the parasphenoid pushes itself between the ethmoidal cartilage and the posterior end of the palatine process causes Dr. Wiedersheim to suggest a doubt as to the correctness of his own theory; and he says that amid these doubts nothing will clear up the difficulty except a knowledge of the embryology of this Urodele.

In my specimens the premaxillary is already well ossified; and there is, even in this early stage, no trace of any original separation into two centres. The alveolar processes are long and comparatively strong. Situated on their border is a number of teeth, eleven, as I count them, one being accurately in the middle line. An examination of the adult in my possession shows that it has the same number and arrangement of the teeth. In the young this median tooth, and one on each side of it, are especially large, sharp-pointed, and directed nearly backwards. There are long ascending and palatine processes, although, as might be expected, they do not extend so far backward as in the adult. The palatine process reaches back nearly to the point where the trabecular cornua diverge from each other. It has no connection with any cartilage, and there is at this stage no cartilaginous nasal septum. It seems quite evident, therefore, that the premaxillary is not a composite structure, but that the palatine process continues to grow backward as a membrane-bone until it attains the dimensions that it has in the adult.

As has already been stated, the anterior lobes of the trabecular cornua, like a divided prenasal process, end in the angle between the alveolar and the palatine processes quite close to the lower border of the latter, and close to one another. To me it now appears quite probable that these cartilages grow

downward until they meet below the palatine process and then coalesce. Afterward, by the expansion medially of the maxillaries and the vomers, the portion of the cartilage below the plane of the vomers becomes cut off from the cornua, and forms the unpaired piece that appears so anomalous. But it will require older specimens than those in my possession to settle this matter fully.

The frontals are long, slender splints which first appear, posteriorly, in those cross-sections which pass through the hinder border of the eye. At their hinder ends they are comparatively thick, and overlap the parietals. They lie entirely above the level of the eyes. At the anterior border of the eyes, the frontals descend to near the level of the cartilage overlying the nasal-sac, the lower edge lying a little nearer the middle line than the upper border of the nasal-sac. They may be traced forward as very thin films as far as the perpendicular section just in front of the divergence of the trabecular cornua. Such sections show the anterior end of the cerebrum, and the hinder ends of the two median processes of the premaxilla. (See Fig. 6.) This anterior end of the frontal passes forward over the olfactory nerve, which is directed laterally into the nasal-sac, and the bone may be followed to the anterior border of the nerve. Wiedersheim (*op. cit.*, pp. 52-53) has shown that the anterior end of each frontal forms a sort of ring or ferrule (*Knochenzwinge*) around the olfactory nerve, and through which this nerve makes its exit from the brain-cavity. According to his descriptions, there is a flat process of bone sent down from the frontal on the outside of the nerve; then in front of this outer process a similar one descends on the inner side of the nerve; then the two are united under the nerve. Already in my specimens the frontal has come into close relations with the nerve, but has not yet enclosed it by means of its processes.

The parietal extends from the perpendicular sections through the hinder border of the suspensorium forward to that passing through the anterior border of the lens. It is better developed than the frontals. Its lower border lies upon the upper border of the auditory capsule and trabecula to the eye, where it rises somewhat above the cartilage. In the latter region also it lies somewhat below the hinder end of the frontals.

Neither the frontals nor the parietals, along their inner or upper borders, approach at all near the middle line.

The vomers, or vomero-palatines, are present as a pair of thin, narrow splints, which extend from the middle of the sub-nasal bands of cartilage backward almost to the antorbital cartilages. They lie parallel with the trabeculæ and a little outside of them. Each is accompanied by a row of dental papillæ, five or six in number. These lie a little to the mesial side of the bone. Some of the papillæ are already undergoing calcification.

There are no deposits of bone to represent the maxillæ; but two rows of tooth-papillæ, five or six in each, which extend backward from the hinder ends of the alveolar processes of the premaxillæ, show where these maxillæ will soon appear.

No bony pterygoids, prefrontals, or nasals are yet to be seen.

The parasphenoid is a broad but very thin and delicate film of bone underlying the brain from just in front of the foramen magnum forward nearly to the coalescence of the trabeculæ, and passing laterally from one trabecula to the other.

The squamosal is a curved bone that overlies the suspensorium and runs upward and backward upon the otic capsule. Its lower border is applied closely to the columella along the anterior half of the latter.

The lower jaw is furnished with two strongly developed bones. One of these is the dentary. It meets its fellow in front to form a symphysis, and extends backward on the outer side of Meckel's cartilage nearly to the articulation with the suspensorium. Arranged along each dentary are about fifteen teeth, only the anterior one of which is anchylosed to the bone. This tooth and the one immediately following it are large and fang-like, and correspond in that respect to the large teeth of the premaxillary.

The second bone of the mandible, the angular, lying along the inside of the cartilage, extends from the angle of the mandible half way to the symphysis.

There is no trace of a splenial bone, and none of an articular.

As before stated, there is a parostosis connected with the ceratohyal. It lies along the inner and lower side of the cartilage, running nearly the whole length of the latter. This slender splinter of bone I have repeatedly been able to dissect off; and having under a cover-glass treated it with hydrochloric acid, have obtained satisfactory effervescence. Wiedersheim (*op. cit.*, Tafel I., Fig. 8) represents the ceratohyal as having a strip of

bone running its length; and an examination of the adult at hand shows that the cartilage is only partly ensheathed by bone.

The lower end of the first branchial arch is meanwhile undergoing ossification of a different kind, being overlaid, as before mentioned, with bone deposited ectosteally.

To the foregoing on the cartilaginous and bony skull, I make the following notes on other structures belonging to the head:—

The common ganglion of the facial and auditory nerves lies wedged in between the otic capsule and the outer bar of the basicranial cartilage. It gives origin, as usual, to the facial nerve, which runs outward to escape by the facial foramen, and to the auditory, which enters and supplies the anterior portions of the labyrinth. Further back the ganglion, or what appears to be a portion of it, seems to be crowded through the mesial wall of the capsule so as to appear to lie partly within the capsule. Here it lies in close relation with the mesial wall of the sacculus, to which it distributes nerve-fibres, as it does also probably to the rudimentary cochlea. This branching of the auditory nerve before it enters the capsule I have observed also in *Amblystoma* and *Spelerpes*. The acoustic nerve in the frog enters the labyrinth by two or more foramina (Owen, *Anat. Vert.*, Vol. I, 312).

The facial nerve, after emerging from the cranial cavity, courses outward and passes below the columella. My sections show this plainly. Dr. Wiedersheim undoubtedly errs when he announces (*op. cit.*, p. 137) the rule that the facial nerve in all Urodeles, without exception, makes its way out over the suspensorio-stapedial ligament, whether this consists of fibrous tissue or cartilage. And unless the relation of the facial nerve to the columella in *Menopoma* is variable, it, too, offers an exception to his rule, despite the figures which he gives to illustrate these parts (*Kopfskelet*, etc., Fig. 24). Messrs. Parker and Bettany (*Morphology of the Skull*, p. 132) state that the cartilage passing between the stapes and the suspensorium lies *over* the facial nerve, and a dissection made by myself is confirmatory of this statement.

Mention has already been made of the foramen of the ductus endolymphaticus. This latter is a narrow tube which enters the brain-cavity, having taken its origin in the sacculus. On the upper and outer surface of the brain it expands into a saccus

endolymphaticus of considerable size ; but those of the opposite sides do not come into contact.

There are at this stage rudiments of two nasal glands. Each consists of a single duct, which opens into the floor of the corresponding nasal-sac and passes directly inward so as to lie finally upon the outer edge of the anterior end of the ethmoidal plate. Here it divides into two tubes, which may be traced for a short distance backward along the inner side of the nasal-sac.

II. THE AXIAL SKELETON.

The vertebræ are undergoing ossification, and this is more advanced in the anterior, than in those of the pelvic, region. The bodies of the vertebræ are invested with a layer of bone which closely surrounds the notochord. Toward the ends of the vertebra the bony sheath expands a little, so that the vertebral body is somewhat hour-glass shaped. There is in the centre of each vertebral body a portion of vertebral cartilage, as represented by Dr. Wiedersheim in his *Comparative Anatomy* as belonging to the vertebra of *Gyrinophilus porphyriticus*. Outside the notochordal sheath, at the ends of each vertebra, is a ring of much-modified intervertebral cartilage. The cartilaginous arches of the vertebræ come down upon the bony sheath of the centra, and the bone rises up from the centra upon these arches two-thirds the distance to their upper ends.

There are no traces of ribs. Above the base of each lateral half of some of the anterior vertebral arches there stands out a process to which the future rib will possibly be attached.

III. THE APPENDAGES.

The shoulder girdle consists of two lateral masses of cartilage, in each of which may be distinguished a scapula, a coracoid, and a precoracoid. The scapula is slender, and is directed downward and forward. The precoracoid is somewhat longer than the coracoid. Both are considerably broader than the scapula. They are widely removed from each other in the middle line below. There is no suggestion of a sternum.

The humerus has its shaft ensheathed in a thin layer of bone.

The ulna and radius, carpal, metacarpal, and phalangeal elements are present in cartilage.

The pelvic girdle consists of a plate of imperfectly differentiated cartilage on each side, which has no connection with the vertebral column above nor with its fellow below. Femur, tibia, and fibula are present in cartilage, as well as some portions of the foot.

IV. OBSERVATIONS ON A LARGER SPECIMEN.

Since the above was written, I have received from the collections of the United States National Museum a specimen of *Amphiuma* six inches long, one of the smallest in the collection. This has been secured in the hope that it might throw some light on the origin of certain structures which had not yet made their appearance in the very young, and might furnish, in the case of other structures, stages intermediate in development between those of the already described larva and those of the adult. Of such structures the most interesting, perhaps, are the unpaired piece of cartilage which is found in the roof of the mouth, and the various portions of the premaxillary bone. The specimen has been decalcified, stained, cut, and mounted serially; and such results as I have been able to obtain are now presented.

As might have been anticipated, this specimen is already too far advanced in development to be of the highest value for the solution of the problems before us. The skull is nearly as thoroughly ossified as it is in the adult. Nevertheless, the preparation is, I think, a very instructive one.

An examination shows that the cartilage which was found in the hinder part of the floor of the brain-case of the unhatched larva has been extensively removed, so that there is now none of this tissue in the middle line between the ethmoidal plate and the narrow basioccipital cartilage. The base of the skull, therefore, as regards the primordial elements, is much like that of *Necturus*, except that such cartilage as remains along the borders of the otic capsules is more extensively ossified in the *Amphiuma*. In the region about the anterior end of the proötic, where in the larva a band of cartilage is sent from side to side, a shelf of bone, now a process of the proötic, extends in-

ward from one-third to one-half the distance to the middle line. On this shelf is supported the trigeminal and facial nerve ganglia. With the central band of cartilage have disappeared also all traces of the notochord from the base of the skull.

The proötics are quite thoroughly ossified. Two points, however, as Wiedersheim has observed, even in the adult, remain in a cartilaginous state, viz. : those to which are articulated the pedicle and the otic process of the suspensorium. A broad band of cartilage, running transversely through the otic capsule in the region of the fenestra ovalis, separates the proötic from the opisthotic. The latter send inward toward the middle line each a process of bone which grows wider as we proceed backward. These, however, nowhere come into contact, but are connected by a considerable basioccipital cartilage.

The foramen magnum is bounded above by the opisthotics, which for a short space come into contact in the middle line. More anteriorly, beneath the hinder ends of the parietals, the opisthotics are separated by a mass of cartilage which may be regarded as the supraoccipital.

The inner wall of the auditory capsule is well ossified. In this inner wall I find anteriorly a foramen for the branch of the auditory nerve, which is distributed to the upper portions of the labyrinth. Further back, a much larger branch of the auditory nerve enters the labyrinth through about three closely placed foramina, and is distributed to the sacculus and probably the lagena. On the inner wall of the sacculus, we find a large macula, and immediately outside of this a very large otolith. (See Fig. 10, *Ot.*) This otolith reminds us of that of some of the fishes. The opening for the escape of the ductus endolymphaticus is situated at the upper border of the wall immediately above the foramina for the saccular branch of the auditory nerve. Just behind the last-mentioned foramina is an opening in the cartilage, as in the larva, through which I have supposed a lymph sinus to pass. This foramen lies just mesiad of the lagena.

The ossification of the trabecula lying mesiad of the proötics is carried forwards, anterior of the foramina for the escape of the fifth nerve. It soon, however, becomes reduced to a mere shell of bone surrounding the cartilage. Then begins the orbito-sphenoidal bone. This is more or less completely ossified as

far forward as the section passing through the lens, at which point the frontals and the vomero-palatines begin to enter into the side walls of the brain-cavity. Contrary to Dr. Wiedersheim's statement concerning the orbitosphenoidal bone in the adult, it is in my specimen higher in front than behind.

Following the trabecular walls forward, we find that before the ossifications have disappeared the cartilage has divided itself into two bars, an upper and a lower, corresponding to those of the larva, which are designated by *Na.C'* and *Tr.* in Figs. 3 and 4. The lower bar is the continuation of the trabecula. Opposite the eye, the upper bar is a very slender rod, which does not lie nearly so far to the outside of the middle line as it does in the embryo, a circumstance due probably to the narrowing of the snout. As soon as the nasal-sac is reached, this rod expands outward, while its inner edge lies against the descending process of the frontal bone. Just where the olfactory nerve pierces the frontal, the cartilage again divides into an inner and an outer portion. The inner division runs forward in the angle between the facial and the descending processes of the frontal and coalesces with the **X** of the cartilaginous nasal septum. The outer division extends forward over the upper outer side of the nasal-sac until opposite the bony internasal septum, where its outer edge unites with the cartilage that underlies the nasal-sac; its inner edge meanwhile extending inward meets and unites with the advancing border of the cartilage that covered the inner and upper wall of the nasal-sac. In other words, we may say that the nasal-sac is roofed over with a cartilage that has in it a large fontanelle, and that this roof mesially coalesces with the cartilaginous nasal septum, while externally it coalesces with the band of cartilage which expands beneath the nasal-sac.

Where the internasal septum is formed by the premaxilla, the cartilage is missing on the inner side of the nasal-sac, but above, below, and on the outer side, the cartilage is unbroken. When the alveolar process of the premaxilla is reached, no cartilage is found immediately over it; but on the outside of the sac and above it, the cartilage continues to the borders of the external nostrils, while just before the nostril is reached, the cartilage is expanded so as almost to surround the passage.

On its lower inner side the nasal cartilage sends a prong into the angle between the body and the alveolar process of the

premaxillary, as has been shown in the case of the larva. Below the premaxillary is found the unpaired piece of cartilage which has already been referred to. There is no cartilaginous connection between it and the processes from the nasal cartilages ending in the angle between the body and the alveolar processes of the premaxillary. Hence, my theory of the origin of the unpaired cartilage is not demonstrated by the specimen in hand. However, it is not disproved; while the apparently transitional character of the intervening tissues is favorable to the opinion that the cartilage has but recently undergone conversion into connective tissue. It is greatly to be desired that a specimen of this species may soon be obtained of intermediate age, so that the origin of this structure may be definitely determined.

The premaxillary has the same features as that of the adult. I can, however, see no grounds for accepting Dr. Wiedersheim's view as to the origin of the median descending plate and the palatine process from the interseptal cartilage. There is nowhere to be seen such a transition from bone to unossified cartilage as might be expected, were the bone derived from the cartilage. The relations between these portions of the premaxillary are no more intimate than is that between these cartilages and the descending processes of the frontals. There is thus no sphenoidal ossification in this animal.

It appears to me that many of the peculiar structures of the *Amphiuma* may be explained by considering its habits. It is eminently a burrowing animal, as has been shown by many observers. Such a mode of life would require and, in time, lead to the production, probably, of a narrow and pointed snout, instead of the rounded snout, so common among the Urodeles. The ability to thrust the body rapidly into the earth at the bottoms of rivers and swamps would also call for a solidly constructed cranium; and accordingly, we find the skull of the *Amphiuma* as thoroughly ossified as in the higher members of its order. In the act of burrowing the premaxillary would be especially exposed to pressure, and it would be essential that this pressure should be transmitted to and sustained by the other bones of the skull. This result is secured in a beautiful and effective manner through the structure and connections of the premaxillary. Its solidity is, first of all, secured by its being composed of but a single piece. At the sides its alveolar

processes are joined to the strong maxillaries, which, instead of being directed widely outward, as in *Cryptobranchus*, are turned backward with their palatine processes lying close to the long and strong vomers. The latter, in their turn, run far back beneath the parasphenoid, and all these bones are firmly bound together by connective tissue. On the upper surface of the skull, too, the maxillary is closely joined to the nasal, and through the prefrontal with the strongly developed frontal. This, however, seems not to be enough. The premaxillary has, above, a process that extends back between the nasals and ends by being wedged in between the frontals for half their length. Below, the premaxillary sends backward a similar spine, which is firmly bound to the vomers and the parasphenoid. The premaxillary is further strengthened by having the two backwardly directed spines connected at their bases by the plate of bone which functions as a partial internasal septum. The whole structure of the skull is in strong contrast to that of the skulls of *Necturus* and *Siren*, both exclusively swimming animals.

Reference has already been made to the peculiar structure of the anterior ends of the frontals, as these have been described by Dr. Wiedersheim. My observations on my largest specimen do not wholly confirm his descriptions. I do not find that the frontal forms anything that can properly be called a ring or ferrule around the escaping olfactory nerve. That the olfactory nerve does leave the brain-case through the frontal is very true. What I do find is this: the anterior ends of the frontals send down each a descending process, which at length touches the ethmoidal cartilage. For a space the processes form the side-walls of the brain-case, and when they have come into contact, they function as a portion of the internasal septum. Where they form the walls of the cranium, the olfactory nerves of course lie mesiad of them. As the processes approach each other like the sides of a wedge, the nerves at length pierce them and enter their respective nasal-sacs. Fig. 7 represents a section made across the head at the point where the nerves are either passing or are about to pass through the frontals. On the right the olfactory foramen has not yet been reached, though the bone is thinning. On the other side the nerve is in the act of escaping through the process. Fig. 8 shows the condition of things only two-thousandths of an inch further forward. Here

we find both nerves on the outside of the descending processes, and yet these processes have undergone no change, except that they have approached each other more closely. These parts may undergo some modifications by the time the animal has reached adult size, so as to justify the distinguished author's description, and his Fig. 20, Tafel II; but it seems more probable that he has been misled by not having closely consecutive sections. With my sections the thousandth of an inch in thickness I have no difficulty in making out the changes in position of the processes. It would almost appear that in the process of lengthening which the snout has undergone the orbitosphenoid and the cartilaginous internasal septum have not been able to keep pace with the other structures, and that their deficiencies have had to be made good in the one region by an extraordinary development of the frontals and in the other by the production of the perpendicular plate of the premaxillary.

Dr. Wiedersheim (*op. cit.*, p. 136) states that "das cartilaginöse Operculum zu einem kurzen ebenfalls knorpeligen Stiel auswächst," etc. My specimen, young as it is, tells a different story. The rim of the operculum is wholly cartilaginous; but both the inner and the outer surfaces of the central portion of it are converted into bone. The head of the columella is coössi-fied to the centre of the operculum; but almost immediately after it has freed itself, the columellar rod becomes cartilaginous. The thickened lower border of the squamosal then descends upon the columella, and is continued upon it to a point somewhat in front of the fenestra ovalis. Here this rod once more becomes surrounded by bone, which passes forward into that of the quadrate. *Sta.* in Fig. 10 points to the bone-incrusted operculum. From the anterior bony portion of the columella a broad process of bone rises up between the squamosal and the otic capsule; and this may be traced backward and upward for some distance, until at length it ends in a point. The termination of this point may be seen in Fig. 10, *Co.p.* For a part of the way anteriorly this process rises nearly to the upper border of the squamosal. Such is seen to be the case in Fig. 9, which passes through the foramen for the facial nerve. Here the axis of the columella is seen to be cartilaginous, but surrounded by bone which passes into a plate lying inside the squamosal. We might say, in other words, that the quadrate

sends upward and backward a strong process between the squamosal and the otic capsule, and that the lower border of this involves the greater portion of the columella. As far forward as the quadrate the squamosal rests on the columella, the process just mentioned springing from the inner border of the columella. This relation is shown in Fig. 9.

If we consider how firmly the quadrate is clamped to the skull by means of the squamosal, how greatly movements of the columella must be restricted by its close connection with the squamosal, and how the backwardly directed process of the quadrate adds to the stability of the parts, we can easily believe that the delicate structures of the labyrinth will be but little disturbed by movements, even the most violent, of the jaws. Since the *Amphiuma*, as has been shown both by the observations of Dr. Shufeldt (*Science*, Vol. II, 163) and myself, attacks its enemies with great vigor, seizing them between its jaws and turning about its long axis like a drill or whirling around in a spiral, it would appear necessary to protect the delicate organ of the ear from such agitation as might during such conflicts be imparted to it through the columella.

The facial nerve is plainly seen to escape beneath the columella. This is shown in Fig. 10, VII.

The principal part of the ossification of the quadrate is found on the outer surface of the cartilage. It is overlapped by the squamosal, and along its outer border sends out a ledge which supports this bone. The remarkable process sent backward by the quadrate has already been mentioned. It may be called the columellar process of the quadrate.

The pterygoid cartilage, which in the larva consisted of a slender rod unconnected with the suspensorium, has now joined the lower border of the ascending process about one-third of the distance back of where the latter unites with the trabecular cartilage. The pterygoid bone is present as a very thin and slender splint, which posteriorly forms a suture with the inner side of the quadrate, and runs forward beneath first the ascending process of the suspensorium and then the proper pterygoid.

Posteriorly the groove for the temporal muscle and tendon is along the lower border of the parietal bone. When the proötic bone is reached, the parietal overlaps it. Farther forward the two bones form a harmonia suture. Near the anterior end of

the labyrinth the proötic rises so as to overlap the parietal, and at length it alone forms the outer wall of the groove. This process of the proötic continues thus to its termination at the very tip of the beak-like process of the parietal, which Wiedersheim has figured on Tafel II, Fig. 17.

The antorbital is almost entirely cartilaginous, but posteriorly coalesces with the ossification of the orbitosphenoid.

I find no cartilage strengthening the capsule of the eye. While such a support seems usually to be present in the eye of the Urodela, I find none in that of *Spelerpes longicaudus*. The integument passes over the eye of *Amphiuma*, and the connective layer is very dense and thick. The animal probably enjoys very limited powers of vision.

In the lower jaw we find the articular undergoing ossification; but this seems to be due rather to an extension of the bone of the angular first around, and then into, the territory of the articular, than to an independent centre.

As already observed in the case of the larva, the ossification of the hyoid seems to be rather a parostosis than a cartilage bone. The bone lies on the mesial side of the cartilage. At the anterior end of the hyoid, the bone seems almost immediately to press itself through the cartilage, so that there is cartilage on both sides of the bone. Further back, the bone thickens, becomes crescentic in section, and partially encloses the outer and main portion of the cartilage. In Fig. 9 at *Ce.h.o.* I have represented the section and position of this bony portion of the hyoid. The letters *Ce.h.c.* point to the portions of the cartilage. These relations continue nearly to the upper end of the hyoid. For the greater distance, the cartilage on the mesial side is a very slender rod, and at one point it disappears entirely, but almost immediately it comes into view again. As there is no trace of this inner rod of cartilage in the larva, it must grow from the extremities of the outer cartilaginous rod. Near the posterior end of the hyoid, the two portions of the cartilage reunite into one mass.

About the ends of the hypohyal are located several nodules of cartilage, which probably represent the basihyal. Dr. Wiedersheim has figured these as he has observed them in the adult.

A large portion of the basibranchial is ossified. The first branchial arch is ossified from end to end. Here, as in the case

of the basibranchial, the calcific deposit forms a shell surrounding the rod of cartilage, but the deposit is also invading the cartilage extensively.

For the opportunity of making my investigations on this extremely interesting animal, I am indebted to Dr. John C. Branner, Director of the Arkansas Geological Survey, and to the liberality of the management of the National Museum.

EXPLANATION OF LETTERS USED IN THE FIGURES.

<i>Ant.</i>	Antorbital.	<i>III.</i>	Oculomotor (?) foramen.
<i>As.p.</i>	Ascending process of suspensorium.	<i>Ma.</i>	Maxillary bone.
<i>Bbr.</i>	Basibranchial.	<i>Na.c.</i>	Cartilage roofing nasal-sac.
<i>B.c.</i>	Middle strip of basicranial cartilage.	<i>Na.s.</i>	Nasal-sac.
<i>Br. I, II, III, IV.</i>	Branchial arches.	<i>Not.</i>	Notochord.
<i>Br.</i>	Brain. [Rhinnencephalon.]	<i>Ot.</i>	Otolith.
<i>Ce.br.</i>	Ceratobranchial.	<i>Pa.</i>	Parietal.
<i>Ce.h.</i>	Ceratohyal.	<i>Pmx.a.</i>	Alveolar process of premaxillary.
<i>Ce.h.c.</i>	Ceratohyal cartilage.	<i>Pmx.n.</i>	Ascending process of premaxillary.
<i>Ce.h.o.</i>	Ceratohyal ossification.	<i>Pmx.p.</i>	Palatine process of premaxillary.
<i>Co.</i>	Columella.	<i>Pr.f.</i>	Prefrontal.
<i>Cond.</i>	Condyle.	<i>Pro.</i>	Proötic.
<i>Co.p.</i>	Columellar process of quadrate.	<i>Ps.</i>	Parasphenoid.
<i>C.t.</i>	Cornu trabeculæ, anterior lobe.	<i>Pt.</i>	Pterygoid.
<i>C.t.'</i>	Cornu trabeculæ, lobe beneath nasal-sac.	<i>S.c.</i>	Semicircular canal.
<i>D.p.</i>	Dental papillæ.	<i>Sq.</i>	Squamosal.
<i>Eth.</i>	Ethmoidal cartilage.	<i>V.</i>	Fifth nerve and foramen.
<i>Fr.</i>	Frontal bone.	<i>VII.</i>	Facial nerve and foramen.
<i>I.</i>	Olfactory nerve.	<i>VIII.</i>	Auditory nerve.
<i>II.</i>	Optic foramen.	<i>VIII.'</i>	Saccular branch of VIII nerve.
		<i>Vo.</i>	Vomers.
		<i>X.</i>	Foramen for vagus nerve.

EXPLANATION OF PLATE II.

FIG. 1. View of two eggs, containing young and showing the connecting cords.

FIG. 2. View of the young taken from the egg, and enlarged to twice the natural size.

FIG. 3. View of the cartilaginous skull of the larva, as seen from above. Enlarged 10 diameters.

FIG. 4. Same skull seen from side. Enlarged as Fig. 3.

FIG. 5. Hyobranchial apparatus. Enlarged 10 diameters.

FIG. 6. Transverse section across the snout of the larva. Enlarged 56 diameters.

FIG. 7. Section across the snout of the larger specimen, six inches long. This is designed to show the relations of the descending processes of the frontal bone to the olfactory nerves. The nerve is seen, on the left side, passing through the descending process. One branch is passing outward to the walls of the olfactory organ. The section is cut somewhat obliquely, so that on the right side the olfactory nerve yet lies mesiad of the descending process. Enlarged 32 diameters.

FIG. 8. Section taken $\frac{2}{1000}$ of an inch anterior to the preceding. Both nerves have passed through the frontals, but the processes continue on with little change. Enlarged 32 diameters.

FIG. 9. Section across head of same individual as the last. Passes through the foramen for the facial nerve. This figure illustrates the ossification of the columella, and the broad process of bone that arises from it and passes up between the squamosal and the proötic. The ceratohyal ossification lying between the two portions of cartilage is seen, as well as the ossification of the first ceratobranchial. Increased 32 diameters.

FIG. 10. Section through same skull and with the same enlargement. It passes through the anterior edge of the stapes. Shows more especially the columella, cartilaginous at this point, the facial nerve passing below it, the posterior extremity of the columellar process of the quadrate, and the passage of a portion of the saccular branch of the eighth nerve into the labyrinth, and the large otolith.

Figs. 5, 6, 7, 8, 9, and 10 were outlined under the camera and the details filled in from the slide under higher power. Figs. 3 and 4 were partly drawn under the camera and partly reconstructed from the sections.

THE SEGMENTATION OF THE PRIMITIVE VERTEBRATE BRAIN.

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PART I.

THE primitive segmentation of the vertebrate brain is a problem which has probably attracted as much of the attention of morphologists as any one of the great, unsettled questions of the day, and many views have been advanced which have, it is true, reached one important point of agreement; namely, that the primitive brain was undoubtedly a segmented structure. But beyond this, in regard to the character of these segments and the number of segments of which the brain originally consisted, I think it can be said with perfect freedom that nothing whatever has been definitely proved. It is the purpose of this paper to add a few more links to the chain of evidence necessary for the elucidation of this important question.

The majority of investigators on this subject have made use of the cranial nerves as a means of determining the number of segments of the primitive brain. Investigations in this line are good as far as they go, but as far as the determination of the original number of segments and the character of these segments by this method is concerned, it is largely conjectural for the following reasons:

1. We have positive proof that the degeneration of certain branches has taken place.¹ This being the case, we have every reason to assume that whole segmental nerves may have once existed, which have completely degenerated, leaving no trace whatever of their previous existence. If such be the case, the segments originally connected with these degenerated nerves must necessarily be overlooked, if the existing nerves are made

¹ Marshall states that the IV nerve possesses a sensory branch in Selachians and Amphibians. Gegenbaur notes the same for Selachians.

use of as a means of determining the original number of segments.

2. Furthermore, the vagrant changes in the position of some of the cranial nerves must necessarily cause confusion. For example, take the VI nerve which in the frog and tadpole stages is situated between the first and second roots of the IX. nerve,¹ a position somewhat posterior to its place of origin. This remarkable shifting clearly shows not only what great changes in position the cranial nerves are capable of undergoing, but it also goes to prove that we can find no reliable means of determining the primitive segments by means of their connection with the exit of the existing cranial nerves. Beard in taking up this problem made use of an important series of sense organs for which he has proposed the name of "Branchial Sense Organs," from their development from thickenings of the epiblast over each branchial cleft. The dorsal branches of certain cranial nerves fuse with these epiblastic thickenings; the superficial part of the thickening giving rise to a branchial sense organ, while the deeper portion becomes the ganglion of the dorsal root of the cranial nerve. This close relation which exists between the dorsal branches of the cranial nerves and their corresponding sense organs is undoubtedly of segmental character. But this line of research is beset by a great difficulty, namely, that the degeneration of certain branchial sense organs would, in time, involve the degeneration of their corresponding cranial nerves, and such degeneration has certainly taken place, in part or in whole, leaving in doubt the primitive segments with which they were connected. As far as I have been able to compare Beard's investigations with my own, I think they are correct when he considers the I., III., V., VII., VIII., IX., and X nerves in connection with their corresponding branchial sense organs, as representing respectively the remains of primitive segments. In fact, my own observations lead me to the same conclusions, but in addition to these I find intermediate encephalic segments between I. and VIII. nerves which Beard's method has led him to pass over entirely.

These investigations were carried on in the Morphological Laboratory of Princeton, under the direction of Dr. Henry F.

¹ I am indebted to Mr. Strong of Princeton for this point.

Osborn, to whom I feel greatly indebted for his kindness in furnishing me with everything necessary for the accomplishment of this work, as well as for valuable advice in connection with it. I also wish to express my thanks to Dr. Henry Orr for the use of his sections of the Lizard. Also to Professor Ryder for some fish embryos which he kindly sent me.

The following types were studied in connection with this subject, which, though not the most desirable, were the only ones obtainable at the season :—

Amphibia, *Amblystoma punctatum*.

Reptilia, *Anolis sagroei*.

Aves, chick embryos.

The general object of this paper is to show that the symmetrical constrictions or folds found in the lateral walls of the embryonic brain are remains of the primitive segmentation of the neural tube, in part atavistic, extending into the primary fore-brain.

Literature.—The folds in the side walls of the medulla or hind-brain have been frequently noticed and commented upon, but only recently has their importance as segmental structures been recognized. Remak in 1850 observed these folds in the medulla, and rightly considered them as structures formed in connection with the “Anlagen” of the cranial nerves. They were observed by Von Baer in 1828 and Dursy in 1869: the latter counted six folds in the hind-brain. In 1875, Dohrn pointed out the segmental significance of these folds with relation to the mesoblastic somites, and in the joint resemblance to the segmentation of an insect embryo. In 1876, Foster and Balfour, and in 1877, Mihalkovics, inclined to give a mechanical explanation to these medullary folds. Béraneck quite recently observed five folds in the medulla of the lizard, and described and figured their connection with the origin of some of the cranial nerves. Kupffer finds in the mid and hind-brains of the trout and salamander at least eight segments, and, if I understand him correctly, says these segments not only correspond to the lateral somites (p. 476), but that there is something similar to these brain segments to be observed in the spinal cord. He concludes, however, by expressing the opinion (p. 477) that the fore-brain is not to be reckoned in the

segmented region. He does not, in his brief paper, give any of the histological characteristics of the segments. I am also indebted to this paper for many bibliographical references.

Gegenbaur has recently expressed the following opinion: "So interessant und so vielversprechend diese Thatsachen sind, so wenig scheinen sie mir gegenwärtig geeignet, zur Beurtheilung der Metamerie des Kopfes selbst als Faktoren in Geltung gebracht zu werden. Das wird erst eintreten können, wenn ihre Beziehung zu anderen, den Kopf aufbauenden Organen erkannt ist."

In 1887, Orr described six folds in the hind-brain of the lizard, five of which are of equal size, and the 6th, from which the 10th nerve originates, somewhat longer than the others. He described the mid-brain as consisting of one fold, and in addition to this described two folds in the primitive fore-brain. He gave the name "neuromeres" to these folds, — a name previously used by Ahlborn with a somewhat different significance. Orr found that the V., VII., VIII., IX., and X. nerves each originated in connection with a neuromere which degenerated after the nerve was formed. He fully described the typical structure of a "neuromere," which I quote, as it bears directly on my own work:

1. "Each neuromere is separated from its neighbors by an external dorso-ventral constriction, and opposite this an internal sharp dorso-ventral ridge, — so that each neuromere (*i.e.* one lateral half of each) appears as a small arc of a circle."

"The constrictions are exactly alike on each side of the brain."

2. "The elongated cells are placed radially to the inner curved surface of the neuromere."

3. "The nuclei are generally nearer the outer surface, and approach the inner surface only towards the apex of the ridge."

4. "On the line between the apex of the internal ridge and the pit of the external depression, the cells of adjoining neuromeres are crowded together, though the cells of one neuromere do not extend into another neuromere."

"This definition of adjacent neuromeres presents, in some sections, the appearance of a septum extending from the pit of the external depression to the summit of the internal ridge (*Spt.*)"

Dr. Hoffmann, of Leiden, published in the *Zoologischer Anzeiger*, June 24, 1889, an article on the segmentation of the hind-brain in the reptiles, which appeared after my abstract of June 14th had been sent to the same journal. (See Bibliography.) He refers to his previous article in Bronn's "Reptilien" (published in 1888), p. 1967, where he considered the hind-brain as consisting of seven metameres or segments, each of which is connected with a nerve in substantially the same manner as described by Orr in his "Embryology of the Lizard," 1887. In his more recent article, as I understand him, he considered the IV. nerve to originate from the first segment of the hind-brain, and to gradually shift its position forward into the mid-brain. I will show that Dr. Hoffmann is probably wrong in considering the hind-brain as consisting of seven segments, and that the segment considered by him as the first segment of the hind-brain is rather the posterior segment of the mid-brain; in other words, it is the second neuromere of the mid-brain (my neuromere Trochlear, Nm. IV.).

In addition to the above statement, Dr. Hoffmann gives the following important evidence in connection with the Trochlear nerve, which I quote in full. "Aus alledem scheint also mit Bestimmtheit hervorzugehen, dass der N. trochlearis einen dorsalen Kopfnerven bildet, denn er besitzt bei Embryonen von *Lacerta* in jungen Entwicklungsstadien ein ziemlich mächtiges Ganglion, welches einen bis unmittelbar an die Epidermis tretenden Fortsatz abgibt, der aber, wie das Ganglion, bald wieder vollständig abortirt, ja es fragt sich selbst. Ob der Nervus trochlearis vielleicht nicht als der vorderste, segmentale Kopfnerv zu betrachten ist, der dem 1, vordersten Segment zuge hört: für diese Meinung spricht auch die Thatsache, dass Ganglion, sobald es sichtbar zu werden anfängt, fast vollständig allein dem 1. Segment aufsitzt, und später auch auf das Mittelhirn übergreift."

From an examination of longitudinal horizontal sections of *Amblystoma*, *Anolis*, and chick embryos, the latter ranging from 30 hours to five days old, I find that the lateral walls of the Myelon and Encephalon (hind, mid, and primitive fore-brain) consist of a series of constrictions which are exactly alike on each side of

the brain; and that the constrictions of the Myelon gradually pass or merge into those of the Encephalon, thereby forming a continuous series of constrictions throughout the entire length of the neuron, which increase in size anteriorly.

For sake of clearness I have classified the constrictions of the neuron as follows:

Constrictions of the Myelon = Myelomeres
 Constrictions of the Encephalon = Encephalomeres } Neuromeres.

The number of encephalomeres¹ actually observed in the types examined is as follows:

	HB	MB	FB
Amblystoma	5		2
Anolis and Chick	6		2

I do not, with Orr, consider the mid-brain as equivalent to a single encephalomere,² but rather relying upon the observations of Kupffer, as equivalent to two (or even three) which have degenerated in the above-mentioned forms, but persist in the Teleosts, and probably in other fishes. The total number of encephalomeres was thus probably ten, divided as follows:

Fore-brain, 2 and possibly a portion of a third.

Mid-brain, 2 or 3.

Hind-brain, 6 or 5.

In order to avoid confusion when speaking of the encephalomeres individually, I have given them names which I think for the present will answer the purpose.

- | | |
|---|---|
| <p>I. <i>Olfactory Neuromere.</i>
 The most anterior neuromere of the primitive fore-brain.</p> <p>II. <i>Optic Neuromere.</i>
 The second neuromere of the primitive fore-brain.</p> | <p>III. <i>Oculomotor Neuromere.</i>
 Mid-brain neuromere.</p> <p>IV. <i>Trochlear Neuromere.</i>
 Second neuromere of the mid-brain (demonstrated in <i>Petromyzon</i>).</p> |
|---|---|

¹ Term proposed by Wilder for the large encephalic vesicles which we cannot now consider in any proper sense segmental. See article "Brain" by Wilder in Reference Handbook of the Medical Sciences, Vol. VIII 8., § 23, prop. X., p. 113.

² For mid-brain neuromeres, see Appendix.

V. *Trigeminal Neuromere.*

The first and most anterior neuromere of the hind-brain.

VI. *Abducens Neuromere.*

The second neuromere of the hind-brain, absent in the Newt.

VII. *Facial Neuromere.*

The third neuromere of the hind-brain.

VIII. *Auditory Neuromere.*

The fourth neuromere of the hind-brain.

IX. *Glossopharyngeal Neuromere.*

The fifth neuromere of the hind-brain.

X. *Vagus Neuromere.*

The sixth neuromere of the hind-brain.

Comparative Structure of the Myelomeres.

The spinal cord is of clearly segmental character, and at a certain period of its embryonic development, at the time of formation of the mesoblastic somites, we see that its lateral walls are constricted in a manner similar to those of the encephalon, and that the transition from the former to the latter is a gradual one.

Gross mounts of chick embryos ranging between 35 and 46 hours old clearly show this structure; also Figs. 4, 4a, which are longitudinal horizontal sections of *Amblystoma*.

I find that the structure of the Myelomeres in the NEWT, LIZARD and CHICK, conforms in every respect to the four characteristics which Orr gives as found by him in the neuromeres of the hind-brain of the Lizard. These four characteristics are quoted in full on a previous page.

An examination of Figs. 1, 2, 3, all of which are camera drawings of neuromeres of the spinal cord, shows—

1. "That the neuromeres have the appearance of small arcs of circles, *i.e.* one lateral half of each (*Nm*). And that the constrictions are exactly alike on each side of the brain."

2. "That the cells are elongated and are placed radially to the inner curved surface of the neuromere" (*in*).

3. "The nuclei are generally nearer the outer surface (*out*), and approach the inner surface (*in*) only towards the apex of the ridge" (*ap*).

4. "On the line between the apex of the internal ridge (*ap*) and the pit of the external depression (*ex*) the cells of adjoining neuromeres are crowded together, though the cells of one neuromere do not extend into another neuromere."

The Relation of the Myelomeres to the Mesoblastic Somites.

The Myelomeres are intersomitic; that is, the centre of each Myelomere is opposite the space between two somites (Figs. 1, 2 and 3). The dorsal branches of the spinal nerves pass from the external surface of the Myelomeres to the space between two somites, which is opposite their point of origin, and fuse with the epiblastic thickenings to form the spinal Ganglia.

Comparative Structure of the Neuromeres.

In the hind-brain of the lizard and chick six neuromeres are distinctly seen. Figs. 5, 5a, 6, and 6a, which in each case are of exactly the same size with the exception of the Vagus Neuromere (*Nm X.*) which is slightly longer than the others. In *Amblystoma*, Figs. 4, 4a, only five neuromeres are found in the hind-brain. The Abducens Neuromere (*Nm VI.*) is not present. The remaining neuromeres are of equal length except the Vagus Neuromere (*Nm X.*) and the Trigeminal Neuromere (*Nm V.*), which are somewhat longer than the others. We have already seen that the Vagus Neuromere in the lizard and chick is somewhat longer than the others, but that the Trigeminal Neuromere does not vary. In *Amblystoma*, the Trigeminal Neuromere is equal in length to about two of the three remaining neuromeres of the average dimensions. (Figs. 4, 4a.)

This variation in size of the Trigeminal Neuromere is due in all probability to the coalescing of the Abducens Neuromere with the Trigeminal Neuromere to form one neuromere. The fact that the recent Amphibia are somewhat removed from the main vertebrate line, and that their development has been influenced by the great quantities of food yolk present, may account in some degree for the varying structure of the Trigeminal Neuromere in *Amblystoma*.

So much for the similarity and points of difference which exist between the neuromeres of the medulla of *Anolis*, *Amblystoma* and the chick, so far as their relations of size are concerned. Now in regard to their histological structure, I find that the four characteristics given by Orr for the neuromeres of the medulla of *Anolis* are represented in every respect in the structure of the neuromeres of the medulla of *Amblystoma* and the chick; that is, the cell arrangement of

the neuromeres in the medulla of all three classes is the same. It has already been shown that the structure of the Myelomeres, in all three of the types studied, conforms in every respect to the typical neuromere of the hind-brain. Thus we see that a conformity of structure exists between the neuromeres of the spinal cord and those of the hind-brain in the three forms studied.

By applying the description given for the structure of the neuromeres in the spinal cord to Figs. 4*b*, 4*c*, 5*b*, and 6*b*, which are camera drawings of the neuromeres in the medulla of *Amblystoma*, *Anolis*, and the chick, it will be seen that the structure of the neuromeres of the medulla and spinal cord in the Amphibia, Reptilia and Aves is identically the same.

Comparative Structure of the Neuromeres of the Primitive Fore-brain.

So far as known to myself, Orr was the first to notice the presence of two neuromeres in the primitive fore-brain of the Lizard, but he did not compare their cell-structure with that of the neuromeres of the hind-brain. In addition to confirming the presence of two neuromeres in the primitive fore-brain of the Lizard, I have also found that the primitive fore-brain of the Newt and Chick consists of two neuromeres. Also between the mid-brain and optic neuromere (*Nm II.*) of the Lizard, Fig. 8*a*, there is a structure (*Nm II.'*) which resembles a portion of a neuromere. Its form is that of an arc of a circle, but the radius of its arc is less than that of either of the two remaining neuromeres of the primitive fore-brain, which I have already said resemble arcs of circles. I make merely a passing mention of this, for the reason that from the existing data nothing but conjecture can result as to its neuromeric value; while on the other hand if it is a neuromere, it ought to be present in toto in some of the lower vertebrates. (See Appendix.)

The fore-brain neuromeres of the Lizard and Chick, so far as their external character and histology is concerned, are true neuromeres. By external character I mean their form and position with respect to each other. Figs. 8*a*, 9, illustrate the following description of the neuromeres in the primitive fore-brain of the Lizard and Chick.

1. One lateral half of each neuromere is an arc of a circle.
2. The elongated cells are placed radially to the inner curved surface of the neuromeres (*in*).
3. The nuclei are generally nearer the outer surface (*out*), and approach the inner surface (*in*) only towards the apex of the ridge (*ap*).

The arrangement of nuclei in the neuromeres of the primary fore-brain does not always conform to the typical structure.

4. On the line between the apex of the internal ridge (*in*) and the pit of the external depression (*ex*) the cells of the adjoining neuromeres are crowded together, though the cells of one neuromere do not extend into another.

The fore-brain neuromeres of the Lizard and Chick persist up to a certain stage in the embryo and finally disappear.

The stage in which the fore-brain neuromeres of the Lizard are fully developed is represented by Fig. 8*a*. In the Chick these neuromeres are prominent in embryos from 36 to 96 hours old. The external character of the neuromeres in the primitive fore-brain of the Newt is not found to be as perfectly developed as those in the Lizard and Chick; that is, each lateral half of a neuromere does not form as perfect an arc of a circle as in the latter, (Fig. 7). I am, however, in doubt whether this variation from the general form is due to the fact that I did not study the stages in which the neuromeres were most fully developed, but rather those in which degeneration had already begun but not been completed. In any case this was unavoidable, as the stages of this species which I possessed were limited to a few. Possibly their development may have been arrested by external means, due to the presence of yolk spherules, which were found present in such great quantities, mixed in among the cells, that it was a difficult task to make out the structure of the neuromeres. It seems probable that one of the above-mentioned reasons may explain this variation of form in the fore-brain neuromeres of the Newt. But that these structures are neuromeres or remains of neuromeres I think there can be no doubt whatever, since their structure in most respects conforms to the typical structure. The cells have a radial arrangement (Fig. 7), and between the neuromeres they are crowded together, but the cells of one neuromere do not enter into another neuromere. The arrangement of the nuclei is variable and does not always conform to the typical one.

The fore-brain neuromeres of the Newt persist up to a certain period and finally disappear, leaving no trace whatever. This we have already found to be the case in the Lizard and Chick.

Up to this point we have seen that the structure of the folds in the lateral walls of the myelon (myelomeres) conforms in every respect to the four characteristics which are found in the hind-brain and primitive fore-brain folds of all three forms studied (with one exception in the Newt), which goes to prove that the encephalomeres are not only remnants of neural segments similar to the myelomeres, but that they were originally continuous.

The mid-brain has been purposely omitted up to this point, but will be considered further on.

Relation of the Auditory Vesicles to the Neuromeres of the Hind-brain.

The importance of this relationship will be seen further on in connection with the nerves of the hind-brain. The auditory vesicle (*aud ves*) in the Newt and Lizard is opposite, in a transverse line, to the auditory neuromere (*Nm VIII.*; Figs. 4, 4a, 5, and 5a). In the embryo Chick it holds the same position as in the Newt and Lizard up to the 96th hour, or slightly later; after this its position is shifted backwards to a point between the auditory and glossopharyngeal neuromeres (Figs. 6, 6a, *Nm VIII.* and *Nm IX.*).

Relation of the Myelomeres and Encephalomeres to their Respective Nerves.

All the neuromeres of the spinal cord give off (on each side) from their dorsal half a mass of ganglion cells, which constitute the dorsal or sensory roots of the spinal nerve (*SpN*), Figs. 1, 2, and 3. In a like manner I find that four neuromeres in the hind-brain and one in the primary fore-brain give rise to dorsal or sensory roots of cranial nerves.

The myelomeres on giving rise to the spinal nerves, in the manner stated above, degenerate soon after the nerves are

formed. The encephalomeres, after giving rise to their respective nerves, likewise degenerate.¹

All nerves mentioned as originating from the centre of a neuromere have reference to the dorsal or sensory root, unless otherwise specified. Orr states that the 1st, 3d, 5th and 6th neuromeres in the hind-brain of the Lizard (neuromeres, *trigeminal*, *facial*, *glossopharyngeal* and *vagus*) give off (on each side) from their dorsal half a mass of ganglion cells which constitute the roots of the V. (VII., VIII.), IX. and X. nerves respectively, and that the 4th neuromere (auditory neuromere) gives off no nerve, but the space opposite to it is occupied by the auditory vesicle. He also states that the VI. nerve arises, though at a much later period than the others, from the ventral portion of the 2nd neuromere (abducens neuromere). Béraneck previously to Orr mentioned the fact that certain of the hind-brain neuromeres of the Lizard held a definite relation to the origin of the V., VII., VIII., and IXth nerves.

My own observations upon the Lizard confirm the above statements of Béraneck and those of Orr in every instance but one; that is, in regard to the origin of the VI. nerve² from the ventral portion of the 2nd neuromere of the hind-brain (abducens neuromere). That it originates ventral to the origin of the other nerves, somewhere *between* the origin of the V. and VII. and VIII. nerves, there is no doubt, but I cannot definitely confirm its point of origin as stated by Orr from the ventral portion of the 2nd neuromere. (See Fig. 5a, which is a camera drawing of the hind-brain neuromeres of the Lizard.) From an examination of this figure it will be seen that the V. nerve arises from the trigeminal neuromere (*Nm V.*); that the abducens neuromere (*Nm VI.*) gives rise to no dorsal root; that the facial neuromere (*Nm VII.*) is connected with the origin of the VII. and VIII. nerves; that the auditory neuromere (*Nm VIII.*) gives off no nerve, but the space lateral to it is

¹ Orr states that the degeneration of the encephalomeres takes place in the hind-brain of the Lizard as soon as the nerve fibres begin to develop. This point I did not satisfactorily make out, either for the neuromeres of the spinal cord or those of the medulla, but I am inclined to think that Orr's statement is correct.

² The preliminary announcement of this paper, which appeared in the *Zoölogischer Anzeiger*, No. 314, 1889, states incorrectly on Orr's authority, that the VI. nerve originates in connection with the most anterior neuromere of the hind-brain. It should read that it arises from the ventral portion of the 2nd neuromere.

occupied by the auditory vesicle; that the glossopharyngeal neuromere (*Nm IX.*) gives rise to the IX. nerve; and that the vagus neuromere (*Nm X.*) gives rise to the X. nerve. I find in the hind-brain of the Chick an exact correspondence in structure to that of the Lizard; that is, in the hind-brain of the Chick the auditory vesicles and nerves hold exactly the same relation to their respective neuromeres as the corresponding auditory vesicles and nerves in the hind-brain of the Lizard do to their respective neuromeres. I think this relationship of neuromeres to nerves has been fully described for the Lizard, and I refer the reader to Fig. 6*a*, which is a camera drawing of the hind-brain of the Chick, that comparisons may be made. We have already seen that the abducens neuromere (*Nm VI.*) is absent in the Newt. I find also, with this one exception, that the remaining neuromeres in the hind-brain of the Newt hold exactly the same relation to the auditory vesicles and nerves as do their corresponding neuromeres in the hind-brains of the Lizard and Chick.

(See Fig. 4, which is a camera drawing of the hind-brain of the Newt, for comparison with the above-mentioned figures of the Lizard and Chick.)

It has been shown in the preceding pages that there are two neuromeres in the hind-brain of the Lizard and Chick, which do not give rise to dorsal or sensory roots (abducens neuromere) (*Nm VI.*) and auditory neuromere (*Nm VIII.*; Figs. 5*a*, 6*a*). It seems probable that these two neuromeres must have once been connected with sensory roots when we consider similar structures in the spinal cord and hind-brain and their systematic connection with dorsal roots. The fact that the abducens neuromere is absent in the Newt may be accounted for in the following manner: namely, that the degeneration of the sensory nerve of this neuromere has resulted in the consequent degeneration of the neuromere itself. But this is pure conjecture, and then the fact still remains, that these two neuromeres have not degenerated in the Lizard and Chick, both of which are representatives of much higher forms than the Newt. Again, the VI. nerve may be the motor element of the primitive segmental nerve of this neuromere (abducens neuromere), its sensory branch having become degenerate. The position of its origin, somewhere between the neuromeres, trigeminal and

facial, may give credence to this view. It is also possible that the VI. nerve is a motor branch of the V. or VII. nerves: the persistence of the VI. nerve and the absence of the abducens neuromere in the *Newt* certainly imply as much.

Most of the early investigators are agreed concerning the origin of the VII. and VIII. nerves from a primitively single trunk, based on the relations of the VII. and VIII. in Mammals. The opposed view of their separate nature has been steadily gaining ground, and I think at present the latter theory has the greater number of supporters. The double nature of these nerves certainly suggests the probability that they were primitively of separate origin, and the following theoretical evidence may throw some light on this theory. The auditory neuromere (*Nm VIII.*) has no nerve connected with it, and it is situated posterior and adjacent to the facial neuromere (*Nm VII.*), which gives rise to the VII. and VIII. nerves. (*Nm VII.*, *Nm VIII.*; Figs. 4, 5*a*, 6*a*.)

The auditory vesicle (on each side of the brain) is situated in the space lateral to the auditory neuromere, (*aud*; Figs. 4, 5*a*, 6*a*), but the dimensions of the vesicle occupy so much of this lateral space, that the space left between the neuromere and the vesicle is very narrow; so narrow, in fact, that a nerve arising from the neuromere could not possibly obtain a growth in it sufficient to perform the functions required of the auditory nerve. Thus it is possible that the VIII. nerve may have been primitively connected with the auditory neuromere before the auditory vesicle became so prominent, and that the gradual growth of the vesicle has pushed it from its original position anteriorly into the facial neuromere, where the fusion of its root with that of the VII. nerve has taken place.

Mid-brain Neuromeres (Nm III. and Nm IV.).

In the *Newt*, *Lizard*, and *Chick* the mid-brain has the appearance of being an enlarged neuromere, larger than any one of the remaining neuromeres of the brain, but equal in size to about three or four of the first five neuromeres in the hind-brain, and not quite as large as the three neuromeres in the primitive fore-brain. Its cell structure is radial, but its nuclear arrangement does not conform to that of a typical neuromere, except that at

its anterior and posterior limits the cells are crowded together and do not enter the adjoining structures. Two nerves are connected with this neuromere of the mid-brain,—the *Oculomotor*, and *Trochlear*,—each of which, according to the recent investigations of Gaskell, conforms to the type of a complete segmental nerve, in that each contains remnants of the primitive sensory elements; that is, they possess “nerve fibres and groups of ganglion cells corresponding in position, and doubtless also in function, with the nerve fibres and nerve cells of the stationary ganglia on the afferent root of a spinal nerve.” Gaskell suggests that both of these nerves (III. and IV.) are probably complete segmental nerves of the type which Balfour supposes to have been the original type, when mixed motor and sensory roots were the only roots present. I do not consider Gaskell’s investigations with respect to the III. and IV. nerves as conclusive without further evidence on the subject, but I agree with him, and on entirely different grounds, that the III. and IV. nerves represent two separate segmental nerves. Taking into consideration the size of the mid-brain neuromere in comparison with the remaining neuromeres of the brain as well as its “neuromeric” characteristics, also the fact that two nerves arise from it, which are probably either two segmental nerves or parts of the same, also the investigations of Kupffer, previously mentioned, in which he states that he found at least eight segments in the hind and mid-brains of the Trout and Salamander, there can be little doubt left but that the mid-brain originally consisted of at least two neuromeres, and that in all probability the III. and IV. nerves were the segmental nerves of these neuromeres respectively. (See Appendix.)

The Primitive Fore-brain Neuromeres and their Nerves.

The optic neuromere (Figs. 7, 8a, 9; *Nm II.*) has no connection whatever with any segmental nerve. The optic nerve is undoubtedly secondary in its nature, and is, I believe, considered by all as outside the series of segmental nerves. It seems probable that the primitive segmental nerve of this neuromere degenerated as soon as the vertebrate eye came into existence, the latter requiring a nerve better suited to perform its functions than the nerve which primitively belonged to the neuromere.

The olfactory neuromere (Figs. 7, 8a, 9; *Nm I.*) is connected with the olfactory nerves, which arise from the neural crest, according to Marshall, in exactly the same manner as the sensory roots of segmental nerves. He also states that the olfactory nerves arise before the cerebral hemispheres, and in the Dog-fish, Trout, Salmon, Axolotl, Frog, Lizard, Turtle, and Chick their development is fundamentally the same.

Orr states that in the Lizard the olfactory nerves spring laterally from the anterior dorsal (nasal) tip of the primary fore-brain, and run a very short distance direct to the nasal thickenings of the epiblast, in which they end. In the Chick it is fundamentally the same. In addition to confirming Orr's statement in regard to the origin and course of the olfactory nerve in the Lizard, I find an exact correspondence in the Newt (Figs. 7a, 7b, 7c). Thus it is seen that in the Lizard, Newt, and Chick the olfactory neuromere (anterior dorsal tip of primary fore-brain) gives off (on each side) a mass of ganglion cells which constitute the roots of the olfactory nerves. This mode of origin, as we have already seen, is exactly the same as that described for the sensory roots in the segmental nerves of the spinal cord and hind-brain. Therefore I think it is safe to say that the olfactory nerve is the sensory division of the segmental nerve which belonged to the olfactory neuromere, which accords with Marshall and Beard, who upon entirely different grounds consider this a true segmental nerve.

General Summary.

It has been my endeavor in the preceding pages to show that a continuous and symmetrical series of folds (neuromeres), increasing in size anteriorly, extend from the lateral walls of the embryonic brain, throughout the entire length of the neuron, and that these neuromeres are the remains of the primitive segmentation of the neural tube.

1. By proving that a conformity exists in the structure of these neuromeres throughout the entire length of the neuron. (See typical structure of neuromeres.)

2. That all of the neuromeres in the spinal cord, four in the hind-brain, and one in the primitive fore-brain, give rise to dorsal or sensory roots.

(a) That the relation of the neuromeres to the origin of their respective dorsal or sensory roots is fundamentally the same in all three regions of the brain in which neuromeres give rise to sensory roots.

(b) That all the neuromeres of the brain, whether giving rise to sensory roots or not, degenerate before the adult stage of the animal is reached.

It has also been shown (see Appendix).

3. That in all probability the mid-brain originally consisted of two neuromeres, and that the III. and IV. nerves were the segmental nerves of these segments.

4. That the number of primitive Encephalic segments was probably ten (six in the hind-brain, two in the mid-brain, and two in the primary fore-brain).

5. That the neuromeres of the spinal cord, opposite the mesoblastic somites, are "intersomitic"; that is, the centre of each neuromere is opposite the space between two somites, or *vice versa*; hence it is seen that nine mesoblastic somites exactly correspond to the nine spaces between ten neuromeres.

It is now a well-known fact that the segmented mesoblast of the trunk extends into the head region, and according to the investigations of Van Wijhe it is there divided into nine mesoblastic head segments, or "Myotomes," as he calls them, which theoretically correspond to the nine spaces between the ten Encephalomes.

Conclusions.

I consider that the primitive vertebrate brain consisted of a series of segments similar to those found in the embryonic spinal cord, and that the encephalomes probably held the same relation to the mesoblastic head segments as the myelomes do to their respective mesomes; that is, they were intersomitic, the centre of each neuromere being opposite the space between two somites and giving off a mixed nerve from the apex.

The region known as the Encephalon is the result of a great differentiation and specialization of the anterior segments of this primitive structure. That differentiation first began and has been the greatest in the most anterior segments, which may account for the greater size of the folds in this region than in

the hind-brain, which, less differentiation and specialization having taken place, naturally conforms more to the primitive vertebrate type. I am aware that the forms examined are insufficient to enable us to reach any positive conclusion in regard to the exact number of segments, but I feel confident that the method which I have adopted is the one by which this vexed question of the primitive segmentation of the head region, both of the neural tube and indirectly of the surrounding mesoblast, will eventually be decided.

In conclusion, I may say that I feel confident that the full number of primitive encephalomeres will be found in Elasmobranch, Ganoid, or Teleost embryos, the investigation of which will form the second part of this paper.

APPENDIX.

The Mid-brain Neuromeres.

Just before sending this paper to the press my attention was called by Dr. Osborn to an article published in the Journal of Morphology by Dr. W. B. Scott, on the Embryology of *Petromyzon*, in which two distinct neuromeres are figured in the mid-brain (Figs. 10, 11). Dr. Scott makes no mention of these structures as having any segmental value, and in Fig. 11 the cell structure shown is evidently purely schematic. My Figs. 10 and 11 are taken from Dr. Scott's plates. The gradual transition of the hind-brain neuromeres into those of the mid-brain is clearly shown in Fig. 10. The gradual transition of the myelomere into the neuromere of the hind-brain in the Newt, Lizard, and Chick has already been mentioned. Thus I think that an examination of various stages of *Petromyzon* embryos will show a continuous series of neuromeres throughout the entire length of the neuron.

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EXPLANATION OF PLATES.

INDEX LETTERS.

- In, IIIn, IIIIn, IVIn.* First, second, third, fourth, etc., cranial nerves.
- Ant.* Anterior.
- Ap.* Apex of internal ridge of neuromere.
- Aud. Ves.* Auditory vesicle.
- Ep.* Epiblast.
- Ex.* Pit of external depression of neuromere.
- FB.* Rudiment of secondary fore-brain.
- HB.* Hind-brain.
- in.* Inner surface of neuromere, or that surface of the neuromere which lines the neural canal.
- MB.* Mid-brain.
- Mes. Som.* Mesoblastic somite.
- Myl.* Myelomere.
- Na.* Nasal thickening of epiblast.
- Nm I.* Olfactory neuromere. First neuromere of the primitive fore-brain.
- Nm II.* Optic neuromere. Second neuromere of the primitive fore-brain.
- Nm II.¹* Possibly a portion of a third neuromere of the primitive fore-brain.
- Nm V.* Trigeminal neuromere. First and most anterior neuromere of the hind-brain.
- Nm VI.* Abducens neuromere. Second neuromere of the hind-brain.
- Nm VII.* Facial neuromere. The third neuromere of the hind-brain.
- Nm VIII.* Auditory neuromere. Fourth neuromere of the hind-brain.
- Nm IX.* Glossopharyngeal neuromere. Fifth neuromere of the hind-brain.
- Nm X.* Vagus neuromere. Sixth neuromere of the hind-brain.
- Nu.* Nuclei.
- Out.* Outer surface of neuromere.
- Op. Ves.* Optic vesicles.
- Spn.* Spinal nerve.
- Spt.* Neural septa.
- Thal.* Thalamencephalon.

All figures of sections have been drawn with the Abbey camera lucida and a Zeiss microscope. Z. 2 A means Zeiss ocular 2, and objective A, etc.

FIG. 1. Longitudinal horizontal section of spinal cord of *Amblystoma*, showing the neuromeres of the spinal cord and their relation to the mesoblastic somites. Also the typical nuclear and cell arrangement. Z. 4 D.

FIG. 1a. Longitudinal horizontal section of spinal cord of *Triton*, showing the same features as in Fig. 1. Z. 4 D.

FIG. 2. Longitudinal horizontal section of spinal cord of *Anolis sagrei*, showing same features as in Fig. 1. Z. 4 D.

FIG. 3. Spinal cord of Chick. Longitudinal horizontal section, showing same features as in Fig. 1. Z. 2 D.

FIG. 4. Longitudinal horizontal section of hind-brain of *Amblystoma punctatum*, showing the five neuromeres in the hind-brain. Also the gradual transition of the neuromeres of the spinal cord into those of the hind-brain; the relation of the auditory neuromere (*Nm VIII.*) to the auditory vesicle (*aud ves*), and the intersomitic relation of the myelomeres to the mesoblastic somites (*mes som*). Also the relation of the neuromeres to the origin of the V., VII., VIII., IX., and Xth nerves. Z. 2 A.

FIG. 4a. Longitudinal horizontal section of a slightly later stage than Fig. 4, in which the neuromeres have begun to degenerate. Z. 2 A.

FIG. 4b. Longitudinal horizontal section of the hind-brain of *Amblystoma punctatum*, in the region of the glossopharyngeal neuromere (*Nm IX.*), showing the typical cell and nuclear arrangement. Z. 2 E.

FIG. 4c. Longitudinal horizontal section of the hind-brain of *Amblystoma punctatum*, between the glossopharyngeal (*Nm IX.*) and vagus (*Nm X.*) neuromeres, showing the cell and nuclear arrangement between two neuromeres (*Spt*). Z. 2 E.

FIG. 5. Horizontal longitudinal section of the hind- and mid-brain of *Anolis sagrei*, showing the six neuromeres in the hind-brain and the relation of the auditory neuromere (*Nm VIII.*) to the auditory vesicle (*aud ves*). Z. 2 A.

FIG. 5a. Longitudinal horizontal section of *Anolis sagrei*, showing the six neuromeres in the hind-brain and their relation to the origin of the V., VII., VIII., IX., and Xth nerves.

FIG. 5b. Longitudinal horizontal section of the hind-brain of *Anolis sagrei*, in the region of trigeminus (*Nm V.*), abducens (*Nm VI.*), and facial (*Nm VII.*) neuromeres, showing the typical nuclear and cell arrangement. Z. 2 D.

FIG. 6. Longitudinal horizontal section of the hind-brain of a five-day Chick embryo, showing the six neuromeres in the hind-brain and the position of the auditory neuromere with respect to the auditory vesicle (*aud ves*). Z. 2 A.

FIG. 6a. Longitudinal horizontal section of a four-day Chick embryo, showing the relation of the six neuromeres in the hind-brain to the V., VII., VIII., IX., and Xth nerves. This section is not cut exactly in a longitudinal horizontal plane. Z. 2 A.

FIG. 6b. Longitudinal horizontal section of the hind-brain of a four-day Chick embryo in the region of the neuromeres, abducens (*Nm VI.*), facial (*Nm VII.*), and auditory (*Nm VIII.*), showing the typical cell and nuclear arrangement of the neuromere facial and the septum (*Spt*) between the adjoining neuromeres. Z. 2 D.

FIG. 7. Longitudinal horizontal section of the primitive fore-brain of *Amblystoma punctatum*, showing the two neuromeres, — olfactory (*Nm I.*) and optic (*Nm II.*). Also the typical cell and nuclear arrangement of the neuromeres. Z. 4 A.

FIGS. 7a, 7b, 7c. Longitudinal horizontal sections through the primitive fore-brain of *Amblystoma punctatum*, showing the origin of the olfactory nerve from the olfactory neuromere (*Nm I.*) and its final fusion with the nasal thickening of the epiblast. These sections are two or three apart. Z. 4 A.

FIG. 8a. Horizontal section of *Anolis sagrei*, dorsal, and parallel to the axis of the primary fore-brain, showing the neuromeres of the thalamencephalon (*Nm I.* and *Nm II.*). Also *Nm II.*, which has the appearance of a portion of a neuromere.

FIG. 9. Horizontal section of a four-day Chick, dorsal, and parallel to the axis of the primary fore-brain, showing the neuromeres of the thalamencephalon. Z. 2 A.

FIG. 10. Horizontal section through the superior portion of the brain of *Petromyzon*. 22 mm. larva. Taken from Dr. Scott's plates on "The Embryology of *Petromyzon*."

FIG. 11. Horizontal section through the anterior portion of the head of a *Petromyzon* embryo just before hatching. After Scott. This section does not show the primitive fore-brain, as it lies at an angle to the mid-brain, due to cranial flexure. The oculomotor and trochlear neuromeres (*Nm III.* and *Nm IV.*) lie anterior to the trigeminal neuromere (*Nm V.*), between it and the primitive fore-brain.

THE LIFE-HISTORY OF THE FORMED ELEMENTS OF THE BLOOD, ESPECIALLY THE RED BLOOD CORPUSCLES.

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I. THE RED BLOOD CORPUSCLES.

THE origin and the fate of the mammalian red corpuscles have been the subjects of an extraordinary number of scientific papers from workers in various fields of biological research. Contributions have been made from the side of pathology, of normal histology, and of embryology, so that to discuss the subject in all its aspects becomes a difficult undertaking. The results of investigation along these different lines are not at all in agreement, so that many theories radically different from one another have been proposed. Indeed, the embryologist, the pathologist, or the histologist often works at the subject without any reference to the results made known by the investigations of the other, inasmuch as the journals in which these results appear are likely to be read only by the specialists in whose interest they are published. To one who reads over the literature even incompletely, the conviction comes, I think, with a good deal of force, that the various phenomena which have been observed and described, and which have served as a basis for the divergent theories, might all find a simpler and better explanation under some one theory. One cannot help believing, in other words, that in the mammalia the method of production of the red corpuscles is essentially the same in disease as in health and in the adult as in the foetus; and furthermore that the formation of these elements takes place not in a number of different ways, but according to some one scheme of reproduction, as in the development of tissue elements in general. Many authors, on the contrary, have advanced one theory of the formation of the red corpuscles as the result of their own work, but have admitted at the same time that the different views advocated by others might hold good under certain

conditions of health or age. On *à priori* grounds it seems to me that most persons will incline rather to the other view, that the production of the red corpuscles takes place in one way under all conditions of life, though in some cases the process may be accelerated or abbreviated, and in others retarded. Inasmuch, then, as decisive proof that the red corpuscles are formed by two or more different methods is wanting, it is allowable to examine critically the different theories which have been proposed, and to endeavor to discover whether the phenomena they are intended to explain cannot be grouped under a common theory. My own investigations have extended over a period of two years; and though they are in many respects incomplete, yet in a number of points satisfactory conclusions have been reached, and it seems to me, as I shall endeavor to show, that the phenomena which I have observed help to some degree at least in reconciling different theories, and indicate that one general plan of formation holds good in all cases.

Since 1838 it has been known that the red corpuscles of the foetal animal are at one period nucleated cells. This was first shown by Rudolf Wagner (1) for the embryo bat and by E. H. Weber (2) in a human foetus of twelve weeks. Most of the observations made upon these nucleated red corpuscles of foetal blood, their occurrence and their relative numbers at different periods of foetal life, we owe to Kölliker (3) and to Paget. Kölliker found that in a sheep's embryo of three and one-half lines the red corpuscles are all nucleated, and Paget makes the same statement from the human embryo of four lines (fourth week). In a human embryo of three months the nucleated corpuscles in the circulating blood make up from one-sixth to one-eighth of the total number of corpuscles, while at five months they are still quite numerous. In a human foetus of this age (five months) which came under my own observation, and which had been born about five or six hours, and was brought to me still enclosed in the amniotic sac, I found that the majority of the red corpuscles were not nucleated, though nucleated forms were still very abundant. Among the nucleated corpuscles some were found with the nucleus fragmented incompletely into a number of pieces, forming what has been

described by Kölliker and by Neumann as the first stage in the disappearance of the nucleus. In foetal cats I have found nucleated red corpuscles in the blood even at birth, though they were few in number. In cat foetuses of an earlier age they are proportionally more numerous. The youngest cat embryo which I examined was one measuring 2.5 cms. from the crown of the head to the root of the tail. A drop of its blood taken from the heart and stained in methyl green (a 1 per cent solution in 0.6 per cent NaCl) showed a number of interesting things which, so far as I know, have not been noticed, or at least not dwelt upon before. In the first place, there were two distinct forms of red corpuscles present in the blood: one, large, oval, and nucleated, resembling somewhat the corpuscles of the reptiles or amphibia. In shape, they were biconcave, irregular, or apparently, in some cases, biconvex, and were so extremely plastic as to appear semi-liquid. When treated with staining reagents, they took on an oval biconvex form. These corpuscles were distinguished, moreover, by the deeper tint of the hæmoglobin which they contained. Their nuclei were variable in size, were usually placed eccentrically, and were characterized by the fact that without exception in the embryo of this age they stained a homogeneous bluish color with the methyl green, showing no trace of nucleoli or intra-nuclear network. The size of these corpuscles in their long diameter varied from about two to four times the diameter of the red corpuscle of the adult mammal. The second form was circular in outline and of the usual size of the cat corpuscles—some of them were nucleated, and some had lost their nuclei (see Fig. 1). The nuclei of the nucleated forms were in some cases stained a uniform green blue color from the methyl green, like the oval corpuscles just described; but in other cases the nuclei showed an intra-nuclear network or granulation. It is worthy of special emphasis that all the red corpuscles which were non-nucleated belonged to this class. Diligent search through a number of preparations failed to reveal a single large oval red corpuscle which did not have a nucleus.¹

¹ After this article was in the hands of the printer, Hayem's extensive work on the blood, "*Du Sang et de ses Altérations Anatomiques*, 1889," came into the author's possession. In it is found a reference to these large corpuscles. He speaks of them as giant nucleated corpuscles, discoid and concave in shape, though occasionally irregular and flat or subglobular.

It is permissible, perhaps, to suppose that the large oval corpuscles represent the form of the red corpuscle characteristic of the ancestors of the mammalia, and to speak of them therefore as ancestral corpuscles, while the smaller circular corpuscles of the usual size of the nucleated red corpuscles of the mammalia exhibit a modification of this ancestral form which has become characteristic of the blood of most of the mammalia. These latter corpuscles under normal conditions lose their nuclei and become changed to the biconcave red corpuscles of the circulating blood, the transition, in the young embryo, taking place in the blood itself. It is not probable that there is any essential difference in the way in which the two forms of red corpuscles are produced; but it is possible that the large (ancestral) form represents an entire embryonic cell, in which hæmoglobin has become developed, while the true mammalian form arises from similar cells after they have broken up by karyokinesis into smaller daughter-cells, in each of which the nucleus is larger relatively to the size of the whole cell. A similar difference in the red corpuscles of the very young embryo has been recorded by Erb (4). He states that, in the blood of two young pig embryos (1 in. long) he found some nucleated red corpuscles of great size and elliptical form, having a superficial resemblance to the red corpuscles of the frog. Most of the red corpuscles had nuclei, and those that had not were always the smaller variety, similar in outline to the corpuscles of the adult animal.

The nuclei of the nucleated red corpuscles of the young embryo (except the larger variety) are lost while in the circulation, and the presumption is that this fate is met by all the truly mammalian red corpuscles. As the embryo grows older and the production of new corpuscles becomes localized in different organs, — liver, spleen, and marrow, — more and more of the early history of the corpuscle is passed over while still in the blood-forming organ, and more and more of the red corpuscles are sent into the blood stream in the non-nucleated stage. After birth, and throughout adult life under normal conditions, we find only non-nucleated red corpuscles in the circulating blood. The nucleated period in their life-history has passed while they were in the blood-forming organ (the red marrow).

Under certain conditions, however, as I shall endeavor to show later, such as severe hemorrhage or anæmia from pathological causes, some of the nucleated red corpuscles escape from the blood-forming organ before the loss of the nucleus has taken place. Neumann (5/2) states that, in the pig, — unlike other mammals, — one can always find in the normal adult animal some few nucleated red corpuscles in the blood. I have met with a similar exception in the opossum. In several animals which I have examined I was able in each case to find a few nucleated red corpuscles in the blood.

The loss of the nucleus is one of the most important events in the life-history of the red corpuscle, and has naturally been the subject of much discussion. It is usually believed, as taught by Kölliker (3), for the embryo, and by Neumann (5/2), for extra-uterine life, that the nucleus disappears within the corpuscle by absorption, which may be preceded by fragmentation. I desire to come back to this subject under another heading, and will not discuss it fully now. It is my belief that the nucleus is lost not by absorption within the corpuscle, but by migration or extrusion from the corpuscle, as shown in Fig. 2. I mention this view at this time to call attention to some evidence in favor of it found in the examination of the blood of the young cat embryos of 2.5 cms. In the blood of this embryo I obtained a number of specimens, such as are shown in Fig. 2, in which the nucleus was seen in the act of passing out of the corpuscle. This appearance has been seen under different conditions by a number of observers, but has usually been explained as a post-mortem change or as the effect of mechanical pressure, action of reagents, or some similar cause. Now, it seems to me that these explanations will not hold in this case, because none of the nuclei of the large oval corpuscles were found extruding, though they were submitted to the same treatment exactly; and, furthermore, amongst the smaller true mammalian corpuscles, only those were found with the nucleus extruding in which the nucleus stained a homogeneous tint with the methyl green. No nucleus showing an intra-nuclear network was ever found escaping from the cell, though there were many such cells in the preparation, and they had passed through the same treatment as the corpuscles with the other kind of nucleus. This last fact, as I shall show later,

holds good for the nucleated red blood corpuscles in the blood-forming organs of cats of all ages, and presumably for other mammals also. In face of these two facts it is hard to believe that the extrusion of the nucleus is in any sense an accidental occurrence. On the contrary, it is the normal means by which the nucleated corpuscle passes into its non-nucleated form.

The place and manner of origin of the first red corpuscles of the embryo have given rise to a number of different theories. Reichert (6) taught that the first corpuscles are formed from the mass of cells from which the heart is developed, the central cells of the mass becoming the blood corpuscles, while an intra-cellular liquid which forms represents the blood plasma. Kölliker (3) afterwards extended this theory so as to take in the great blood-vessels as well as the heart. He believed that the first blood corpuscles of the embryo are colorless nucleated cells like the other embryonic cells, and at first are found in the solid cords or masses from which the heart and first blood-vessels are developed. The peripheral cells form the walls of the blood-vascular organs, while the central cells are floated away in the plasma which forms between the cells. The red corpuscles are spherical nucleated cells which multiply by division in the blood stream. Within recent years a somewhat similar view has been proposed by Ziegler and by Wenckebach. Wenckebach's (7) first observations were made upon embryos of *Perca fluviatilis*. These embryos are so transparent that they can be examined entire under the microscope. Ziegler (8) worked chiefly with the salmon. Both state that the heart begins to beat and forces into circulation a colorless plasma before the red corpuscles appear. These are seen later, and are formed first, according to Wenckebach, from a mass of cells lying in cross-section between the aorta and intestine, and outlining the position of the future posterior vertebral or cardinal vein. The blood plasma, percolating through this mass of cells, washes off the central ones, to form the first red corpuscles, while the peripheral cells form the walls of the future vein.

Ziegler also describes a mass of cells found in cross-section between the notochord and intestine, which he calls the intermediate cell mass. The central cells of this mass become the first blood corpuscles in the way described, while the peripheral

cells form the walls of the single median cardinal vein, which anteriorly splits into the two posterior cardinal veins. Moreover, from this central mass of embryonic corpuscles cords of similar cells branch off toward the yolk on either side, forming the outlines of new blood-vessels (veins), and serving as centres of origin for new red corpuscles. Both authors believe that these embryonic blood corpuscles are capable of multiplication, since karyokinetic figures are not unfrequently seen. The first red corpuscles, according to this view, are true mesoblastic cells, set apart for the formation of certain veins as well as red corpuscles. Most English embryologists, on the contrary, adhere to the view proposed by Klein (9), Balfour (10), etc., a general account of which is given in Foster and Balfour's *Embryology of the Chick*. According to this theory, the blood-corpuscles are formed endogenously within large mesoblastic cells found chiefly in the area vasculosa and area pellucida. These cells send out processes which unite, forming thus a protoplasmic network. At the nodal points of this network the nuclei of the original cells multiply, to form groups. The protoplasm around each of these assumes a red color from the development of hæmoglobin, and the groups eventually break up to form nucleated red corpuscles. By a similar method, blood corpuscles are formed in the connecting processes, while some of the protoplasm remains granular and uncolored, forming the walls of the future vessels. The nuclei scattered along the walls are also derived, like the corpuscles, from the nuclei of the original mesoblastic cells. The plasma in which the corpuscles float is a secretion from the protoplasmic walls of the newly formed vessels.

Gensch (11) in a communication giving the results of some work done under Kupffer upon the teleosts (*Esox lucius* and *Zoarces viviparus*) states that the first blood corpuscles develop out upon the yolk beyond the boundary of the mesoblast. In this region there is a layer of large plasmodium like cells lying beneath the epiblast, but not forming a continuous epithelium. The cells become united to one another by processes, and the blood corpuscles are constricted off from them, forming the blood islands seen in the blastoderm. This layer of formative cells is called by Kupffer the "secondary endoderm." The theory differs from those previously mentioned not only in the

way in which the first corpuscles are formed, but also in the fact that the original cells are not derived from the mesoblast. In this last respect Kupffer's theory bears some resemblance to the well-known parablasic theory of His (12). His believes that the mesoderm in the sense of the term used by Remak can be separated into an axial and a peripheral portion. The axial portion is formed in the higher animals in the neighborhood of the primitive streak; in the lower animals, in the groove of the blastopore. From it are derived the muscles, the chorda, the generative epithelium, the duct of the pronephros, etc. It falls into two layers, which taper off laterally, but do not extend beyond the body proper of the embryo. The peripheral portion of the mesoderm forms what His calls the parblast; and, when first formed, it lies outside of the body of the embryo, arising in fact from the white yolk. The parblast gives rise to the blood corpuscles and blood-vessels as well as the general connective tissues. Though arising outside of the body, it afterwards grows in from the periphery, penetrating the germ layers, so that the parablasic cells become inextricably mixed with the cells of the germ layers.

To which of these various theories the balance of evidence tends it is impossible to say. In my own work I have not attempted at all to follow the development of the first corpuscles in the germ layers of the embryo. If we suppose that the method of formation of the first red corpuscles in the germ layers is similar to that which prevails in later embryonic life and in extra-uterine life, then it seems to me highly probable, for reasons which I will give presently, that the general method described by Reichert and Kölliker, and afterwards extended and modified by Wenckebach and Ziegler, is most worthy of belief. According to this view, the primitive blood corpuscles form one variety of mesoblastic cells, which become arranged in masses or strings that mark the position of future blood-vessels (veins). The central cells become red blood corpuscles; the peripheral cells form the walls of the vessels.

Development of the Red Corpuscles in the Later Stages of Embryonic Life.

Kölliker (3) first proved that the liver, as soon as it is formed, becomes the seat of production of new red corpuscles. This

fact has been abundantly confirmed by all observers since Kölliker's time, and is capable of easy demonstration. The way in which the red corpuscles develop in the liver has not, as far as I know, been described in any detail. Kölliker held simply that the liver contained certain nucleated white corpuscles which become transformed to red corpuscles by the development of hæmoglobin, and which subsequently lose their nuclei. Neumann (5*d*) states that he finds in the liver nucleated red corpuscles in greater numbers than can be accounted for by supposing that they are carried there by the splenic veins and other vessels opening into the liver. They must be formed in the liver then *de novo*, and he suggests at least two methods by which they are produced. First by endogenous formation in certain large cells. A number of nuclei arise in these cells by a process of endogenous division, and a homogeneous yellow substance collects round each nucleus. Each nucleus with its surrounding colored protoplasm constitutes a red corpuscle, and this is afterwards liberated and undergoes its further development. In addition he finds in the embryonic liver a number of free nuclei which are undoubtedly the same in structure as the nuclei of the nucleated red corpuscles. How these free nuclei arise, and what becomes of them, he leaves undescribed, but supposes that they represent one step in a second method of production of nucleated red corpuscles. He seems to suggest, indeed, that the free nuclei form round themselves a protoplasmic envelope in which hæmoglobin is afterwards developed, and in this way they are converted to nucleated red corpuscles,—a view which, as we will see, has been proposed by others to account for the formation of new corpuscles in the marrow in post-natal life. Neumann says, moreover, that a new development of capillary blood-vessels is taking place in the liver throughout almost the whole of embryonic life, and in some way the formation of the new red corpuscles is connected with the existence of these newly forming blood-vessels. Foa and Salvioli (13) believe that the nucleated red corpuscles found in the liver are derived from colorless corpuscles,—“hyaline cells,”—which in turn arise by constriction or segmentation from the large giant cells found in the liver. It is undoubtedly true that in the embryonic liver the nucleated red corpuscles are formed from colorless cells. Whether one studies

the liver from sections or from teased specimens, he finds abundant proof for this in the transitional forms, which occur in considerable numbers. With reference, however, to the origin of these colorless cells, I cannot agree either with Neumann or with Foa and Salvioli. In sections of liver in the later periods of embryonic life one finds the nucleated red corpuscles and their colorless predecessors lying between the rows of liver cells, scattered irregularly, and without any very apparent relationship to the other elements of the liver. But in the earlier periods of embryonic life, — in the embryo, for instance, 2.5 cms. long, — sections of the liver show the origin of the blood corpuscles quite distinctly. One sees in such sections that the blood-forming cells are not scattered without order, but are grouped into cords or strings lying between the columns of true liver cells which are just beginning to show a typical structure and arrangement. The cords of blood-forming cells resemble those described by Wenckebach, Ziegler, etc., in the germ layers of the young fish embryo, and here also undoubtedly mark out future blood-vessels. One often sees the solid mass of cells stop more or less suddenly while the channel becomes filled with coagulated plasma containing here and there fully developed red corpuscles with or without nuclei. A drawing showing the appearance described is given in Figs. 13 and 14. I have obtained similar cords of blood-forming cells, evidently developing blood-vessels, in longitudinal sections through the posterior limb of the same embryo, as shown in Fig. 15, which indicates that though the production of red corpuscles at this time is most active in the liver, it is also going on in other parts of the body, probably wherever new blood-vessels (veins) are forming. If we accept the theory proposed by Kölliker, Wenckebach, and others, as to the method of formation of the first blood corpuscles in the embryo, then their production in later embryonic life is seen to follow essentially the same plan. One might suppose, indeed, that the cords of blood-forming cells in the young liver are directly or indirectly derived from the original median mass of blood-forming cells which first appears in the embryo, though I have no observations at all which can be taken as evidence for such an hypothesis. A similar method of origin of the red corpuscles in the marrow of birds during extra-uterine life has

recently been described by Denys (14), as I shall have occasion to mention later on.

During the second half of intra-uterine life the spleen also takes part in the formation of red corpuscles. This was first made known by Kölliker, and has been confirmed by a number of observers since his time. In the foetal cat the sequence in which the blood-forming organs enter upon their function is as follows. First, the liver; then, as the production of new red corpuscles in the liver becomes diminished, the function is taken up by the spleen. So that at the time of the maximum activity of the spleen the liver takes but little part in the process. Still later in embryonic life, after the long bones of the limbs have been formed, one finds that the young marrow has begun to produce new red corpuscles, while the activity of the spleen in this respect has suffered a decided diminution. Shortly before birth it is easy to prove that at least three organs are taking part in the formation of red corpuscles, — namely, the liver, the spleen, and the bone marrow, — and even after birth for a certain short time the same is true; in the cat, for as long as three or four weeks. Later than this, however, nucleated red corpuscles showing signs of active multiplication are found only in the red marrow of the bone. The liver certainly takes no part at all after this time in the formation of red corpuscles; and in the spleen one finds under normal conditions no indication of the presence of nucleated red corpuscles. Whether or not the spleen plays any part in the formation of the colorless cells from which the nucleated red corpuscles are afterwards produced will be discussed later.

While it must be accepted that during embryonic life red corpuscles are made in the three organs mentioned, it seems to me quite certain, also, that they are formed during this period in other parts or organs of the body, — wherever, in fact, developing blood-vessels (veins) are found, — though I have no evidence for this other than the section already described, which passed through the long axis of the posterior limb and showed a developing blood-vessel with its young corpuscles lying in the muscular tissue. In describing the development of the red corpuscles at this period in the life of the animal, — that is, just before and shortly after birth, — mention should be made of the discovery of the vaso-formative cells by Ranvier

(15) and by Schäfer (16). Both of these observers have found in the foetus, or in the new-born mammal (rat), large connective tissue cells, within which red corpuscles are produced endogenously. The cells become elongated, and connect with one another to form capillary blood-vessels. The newly formed corpuscles are never nucleated, and in this respect differ from the corpuscles produced endogenously in the germ layers of the embryo according to Balfour and others. In his recent book, Hayem confirms the work of Ranvier, and states in addition that blood plates as well as red corpuscles can be seen in the vaso-formative cells. The hæmatopoietic value of these cells cannot be very great, as they have not been found at any other period in the animal's life, except at birth or shortly afterward. It seems to me that more extended observations are needed before we can accept such a peculiar method of production as one of the normal means by which new red corpuscles are formed. Most of the recent work has shown that the red corpuscles pass through a nucleated stage, and are not formed endogenously within larger cells, so that the isolated observations even of such distinguished histologists cannot be weighed against the combined work of so many other investigators. Possibly the appearances upon which the theory is based may be capable of another explanation.

The White Corpuscles and Blood Plates during Embryonic Life.

I have little that is new to add to our knowledge of these two elements of the blood during embryonic life; but the little I have is worthy, perhaps, of being placed upon record, especially as it is a subject which seems to have attracted very little attention and about which our information is deficient. In the youngest embryo which I examined (cat, 2.5 cms. long), no ordinary white corpuscles could be found, though the blood was thickly crowded, of course, with nucleated and non-nucleated red corpuscles. Occasionally a colorless corpuscle was found; but these differed so much from the usual white corpuscle of the circulating blood, from both the uninucleated and multinucleated form, that it seemed probable that they did not belong to the class of leucocytes, but were embryonic cells which had got into the blood accidentally, either in opening the

heart or in some other way. In any case, they were extremely few in number, and did not resemble the white corpuscles of the grown animal. The blood plates also were entirely absent from the blood of this embryo. Not a single specimen could be found, though a number of preparations were examined. It seems to me that this fact has a bearing upon the theories of the origin of this element, and I shall refer to it again when discussing that subject. In a human embryo of five months, both white corpuscles and blood plates were found, though both were present in small numbers. The white corpuscles were of two kinds, as in the adult, — one variety of small size, with a single vesicular nucleus resembling the lymphocytes; and the other of larger size, faintly granular, with several nuclei, or, more correctly, with a fragmented nucleus. At this age in the human embryo, the great majority of the red corpuscles have lost their nuclei. In a cat embryo of 9 cm. length the leucocytes and blood plates were both found, though the former were present in small numbers. I have not been able to find any special reference to the occurrence of these elements in the foetal blood, except in a paper by Neumann (5*d*). In the examination of human foetuses made by Neumann, he states that generally the white corpuscles were very few in number, but makes no reference to the variations with the age of the foetus. The fact that the white corpuscles are so late in appearing is important, not only in its bearing upon the old theory that they become changed into red corpuscles, but also in the fact that it furnishes a means of determining their influence upon the chemical composition of the plasma.

II. FORMATION OF RED CORPUSCLES DURING EXTRA-UTERINE LIFE.

Historical Review.

The greater portion of the literature of the red blood corpuscles bears upon this side of the subject. Very many different views have been proposed; and a brief presentation of the most important of them may be of value, both as an indication of the drift of opinion upon the subject, and also to enable me to present my own work afterwards in the briefest possible way, without detailed reference to or comparison with other

views. I shall not attempt to make the review as complete as the material which I have accumulated might enable me to do, since some of the work published does not need special mention. I have added, however, an appendix, giving the complete list of articles which I have been able to consult.

Before 1869 it was quite generally believed that the red corpuscles are formed from the white corpuscles, most probably while in the circulation. This theory found its way into the text-books, and, to a certain extent is still advocated by some histologists. In fact, some of the most recent investigations favor this view, although the evidence is so overwhelmingly against it.

Feuerstack (17) in a recent series of observations, made upon animals with nucleated red corpuscles, describes in the circulation transitional forms between the white and the red corpuscles. The colorless cell from which the other forms are derived has a relatively large nucleus and small cell body. The cell substance increases while the nucleus becomes smaller, and not unfrequently takes a peripheral position. Hæmoglobin develops in the cell, which gradually changes in shape from a spherical to an oval form. Most of the transitional stages are found in the bone marrow and spleen, though in these organs they occur not in the parenchyma, but within the blood-vessels. Feuerstack gives no sections to show how these developing corpuscles are placed within the blood-vessels of the marrow and spleen. When he says that the red corpuscles are derived from white corpuscles, he differs somewhat from the older observers, who thought that the white corpuscles might change to red anywhere in the circulation,—were, indeed, continually undergoing such a change,—while Feuerstack limits it to the blood-vessels of the marrow and spleen. On the other hand, his conclusions, if not taken too literally, agree very well with some new views of Denys upon the formation of red corpuscles in the marrow of birds. While the older observers accepted this view of the origin of the red corpuscles without much question, no one was able to obtain satisfactorily the transitional forms, so that Kölliker (3) was forced to say that the question was still undecided. Erb (4) asserted, however, that he was able to get transitional forms in the circulation by means of a certain method of treatment. Blood after treat-

ment with acetic or picric acid gave him peculiar red corpuscles which contained fragments or granules of what appeared to be nuclear matter. These granules might be many or few in number and varied greatly in size. He believed that the cells represent the transitional forms between the white and red corpuscles, and thought that they were more numerous after a severe hemorrhage, during the period of regeneration of the blood. In rabbits, moreover, after a starvation of seven to nine days, the transitional forms could no longer be found. His complete theory of the origin of the red corpuscles is as follows. White corpuscles arise in the spleen and lymph stream and get into the blood first as small uninucleated cells, with a scanty cell substance. While circulating in the blood, these leucocytes increase in size, the increase affecting both the nucleus and the cytoplasm. The nucleus then begins to fragment, and finally breaks up into granules, while hæmoglobin develops in the cell, making thus one of his transitional forms. The fragments of nuclear matter gradually disappear; the cell becomes smaller and takes the shape of a normal red corpuscle. The transitional forms of Erb can undoubtedly be found in the circulation under certain conditions, but Erb was in error in believing them to form an intermediate stage between the ordinary white corpuscles of the blood and the red corpuscles. Their real significance I shall describe later. A number of other theories proposed during this period found but little support. For instance, Wharton Jones (18) thought that the red corpuscles are the liberated nuclei of the white corpuscles, but seems to have had no stronger reason for this belief than an alleged agreement in size. Gerlach, Funke, Schaffner, *et al.*, taught that the red corpuscles are formed endogenously within certain large colorless cells found in the spleen. But the cells containing red corpuscles, which they found, and upon which the theory was based, were afterwards shown by Kölliker to be not the mother-cells of the red corpuscles, but, on the contrary, their destroyers. At present, there can be no doubt that the white corpuscles of the blood are never transformed into red corpuscles, though it must be borne in mind that this does not mean that the red corpuscles of the blood are not derived from white or colorless cells. On the contrary, as we shall see, it is now the general belief that the red cor-

puscles spring from colorless cells found in the blood-forming organs ; but these colorless cells are not the white corpuscles of the blood, indeed never under normal conditions get into the circulation.

The most important discovery of the century with reference to the development of the red corpuscles was made simultaneously and apparently independently by Neumann (5*a*) and by Bizzozero (19*a*). In 1868 these observers found that in the red marrow of the bone nucleated red corpuscles occur, which are similar to those found in the embryo, and that they are present throughout the life of the animal. A nucleated red corpuscle can only be interpreted as the predecessor of a non-nucleated red corpuscle, and the discovery therefore meant that the red marrow of the bones is an organ for the production of new red corpuscles throughout extra-uterine life. In his first papers Neumann spoke of the nucleated red corpuscle as being derived from colorless lymphoid cells, and described transitional forms ; but in his later papers he does not lay so much stress upon the transitional forms, while still believing without doubt that the red cells are derived from colorless ones. With reference to the change from the nucleated red corpuscle to the ordinary form, he agrees practically with Kölliker's view of the nature of this change in the embryo. The loss of the nucleus takes place by a process of absorption within the cell, and may be preceded by fragmentation. Neumann (5*b*) believes that in the adult the bone marrow is the sole organ for the production of new red corpuscles, and gives a number of experiments to prove that the spleen takes no part in their formation, either under normal conditions or after severe hemorrhage. Bizzozero also found the nucleated red corpuscles in the marrow, and afterwards showed that these cells are capable of multiplication by indirect division. This latter observation has been confirmed abundantly by later investigations, and makes a second important step in our knowledge of the origin of the red corpuscles. Bizzozero placed too much weight apparently upon this growth of the nucleated red corpuscles, and overlooked the importance of the colorless cells from which the red corpuscles arise. The nucleated red corpuscles of the marrow are derived, he thinks, from the similar embryonic cells occurring in the liver and spleen during foetal life. When

these organs begin to lose their hæmatopoietic function, some of the nucleated red corpuscles found in them are carried in some way to the marrow, where they form centres of growth for similar cells throughout life. Bizzozero, like Neumann, thinks that the red marrow alone possesses this function during extra-uterine life, but, unlike Neumann, he believes that the spleen may temporarily resume its blood-forming functions after severe hemorrhage when the marrow alone is unable to regenerate new corpuscles with sufficient rapidity. As evidence for this statement he publishes experiments made by himself (19*c*) and also in connection with Salvioli, in which it was shown that, with dogs and guinea-pigs, nucleated red corpuscles can be found in the spleen of the adult if the animal has been subjected previously to a severe bleeding, or, better, to a number of successive bleedings: under such conditions, not only were the simple nucleated forms found, but nucleated cells in process of division by karyokinesis. In man, also, after death from anæmia there are several cases recorded in which nucleated red corpuscles have been found in the spleen (Foa [22], Pellacani). On the other hand, Neumann (5*h*) contends that even after severe hemorrhage nucleated red corpuscles are not found in the spleen, or are found in such small numbers that their presence may be accounted for by the fact that they occur also in the circulating blood, especially in the vena azygos, which brings back blood from the red marrow of the ribs. With reference to this last point, it is undoubtedly true that in the blood of animals after severe and repeated hemorrhages, nucleated red corpuscles may be found, and similarly in the human subject it is known that in pernicious anæmia, leukæmia, etc. (Osler and Gardner [23], Laache [25]), nucleated red corpuscles may be found in the circulation. But it must be borne in mind that in the spleen of animals after strong hemorrhage one may find nucleated red corpuscles in cases when they are absent from the general circulation; and furthermore they may occur in the spleen in large numbers, and showing every sign of an active multiplication. Neumann (5*h*) himself admits that in one case in which the animal (dog) had been bled a number of times, and in which septic infection had developed, he could find nucleated red corpuscles in the spleen, but not in the circulating blood. He

accounts for this exception by attributing it to the septicæmia; but this does not seem to be a satisfactory explanation. Several experiments of my own on this point I will describe in the proper place: it is sufficient to say here that they confirm the view of Bizzozero and others that the spleen may be made to resume its hæmatopoietic activity.

While the fundamental discovery of Neumann and Bizzozero has been generally accepted so far as it fixes the function of producing red corpuscles in the marrow, a number of observers have differed from them and from one another as to the method by which the red corpuscles are formed in that organ. Rindfleisch (26) describes the nucleated red corpuscles, but differs from all others in believing that these cells lose their nuclei not by a gradual absorption, but by an extrusion. Rindfleisch is generally quoted as saying that the nucleus is extended naked from the corpuscle; but this is an error. He says that "the nucleus, surrounded by some colorless protoplasm, leaves the cell, which remains as a bell-shaped body of a reddish yellow color." The further fate of the extruded nucleus, with its envelope of protoplasm, he leaves undiscussed. He describes and figures the nucleus in the act of escaping, but was not able to watch the process in a living cell, though he used all sorts of means—heat, electricity, reagents of different kinds—to act upon the corpuscles. After the extrusion of the nucleus, the red corpuscle has first a bell shape, and is afterward moulded into a biconcave disc by the movement of the circulating blood. Malassez (27) believes that the nucleated red corpuscle is derived from an undifferentiated marrow cell, which contains little or no hæmoglobin, and in which the nucleus is diffuse. He describes three intermediate stages in the transformation which he is able to recognize constantly in the marrow: 1. Spherical cells of large size, which stain very feebly with eosin or hæmatoxylin, and contain no hæmoglobin, or only a trace. The nucleus in these is not a distinct morphological structure, the nuclear matter being diffused throughout the cell. He designates these cells as protohæmatoblasts.

2. Cells of the same size, with a granular protoplasm, and still containing little or no protoplasm. A nucleus is now present, and is spherical, large, and uniformly granular.

3. Cells of smaller size, containing hæmoglobin. The nucleus, also, is smaller, and shows a reticular structure. The next stage is the nucleated red corpuscle proper, which differs from (3) in the deeper tint of the hæmoglobin and the smaller size of the nucleus. The nucleus is distinguished further by the fact that it stains more deeply with hæmatoxylin. Malassez differs from other histologists in his explanation of the way in which the ordinary non-nucleated red corpuscle is derived from the nucleated form. According to him, the latter do not lose their nuclei at all, but give rise to the ordinary red corpuscles by a process of budding. The buds are constricted off, and are first spherical, but afterward become biconcave, partly from the mechanical action of the circulating blood, partly because of an unequal diminution in bulk. Foa and Salvioli (13) also derive the nucleated red corpuscle from a colorless or "hyaline cell." This latter cell originates both in the embryo and the adult from the large giant cells found in the marrow during extra-uterine life, and in the liver and the spleen of the fœtus during the period when these organs are producing red corpuscles. The giant cells, myeloplaques, of Robin are of two kinds, at least, in the red marrow. One variety is large, finely granular, and contains a number of oval separate nuclei, which correspond to the myeloplaques as usually described. The second variety is not multinucleated, but has a very large coiled, or twisted, nucleus, made up, apparently, of a number of smaller nuclei, which are, however, still in connection with one another. This variety Bizzozero described as the "giant cell with budding nuclei"; and Foa and Salvioli believe that they give rise to the hyaline cells, from which the nucleated red corpuscles are afterwards formed. They give to this kind of giant cell, therefore, the name of hæmatoblast. The hæmatoblasts separate into a number of smaller hyaline cells, the large nucleus breaking up into separate "buds," each of which becomes the nucleus of a hyaline cell. The hyaline cells change to nucleated red corpuscles by the development of hæmoglobin within the cell substance; and these latter pass to the non-nucleated form in consequence either of an absorption or an extrusion of the nucleus. In a later paper, Foa (22) expresses his belief that the nucleus disappears within the cell by absorption. Osler

(28), in his Cartwright lectures, describes in the adult marrow seven different kinds of cells. He derives the nucleated red corpuscles, in the first place, from a colorless cell 9 to 12 μ . in diameter, with a smooth, homogeneous protoplasm and a finely granular nucleus. This cell shows, moreover, a peculiar flexibility. These cells, in turn, are derived from what he calls "protoleucocytes," which are solid-looking lymphoid elements, 2.5 to 5 μ . in diameter, resembling free nuclei, though some of them may have a narrow rim of protoplasm. The nucleated red corpuscle is transformed to the non-nucleated corpuscle by the gradual disappearance (absorption) of the nucleus, after which the corpuscle becomes condensed to the flattened disc shape.

The most elaborate, and probably the most important, contribution to our knowledge of the development of the red and the white corpuscles which has been made recently is found in a series of papers by Löwit (29). The most important conclusions at which he arrives are as follows: the blood-forming organs of the adult, among the cold-blooded as well as the warm-blooded animals, are the bone marrow, the spleen, and the lymph glands. In all of these organs we meet with two kinds of colorless cells. One of these he calls "leucoblasts"; and they are destined to form the leucocytes of the blood and lymph. To the second he gives the name of "erythroblasts": from these the red corpuscles are developed. These two sorts of cells are distinguished from each other by differences in the structure of the nucleus, in the method of multiplication, and in the properties of the cell protoplasm. The leucoblasts have a nucleus which is relatively quite large. It contains one or more small heaps of chromatin, sometimes irregular in shape, from which a system of delicate lines and bands radiates toward the nuclear membrane. This latter consists of a distinct, often doubly contoured, band of chromatin substance, on the inner side of which one frequently finds irregular projections connected with the intra-nuclear network. The leucoblasts multiply by a process less complicated than ordinary karyokinesis and more complicated than simple direct division. The chromatin granules during division show some movement, though of an irregular character, from the equator toward the poles. He proposes to call this *divisio per granula*. The erythro-

blasts have a nucleus which shows always a chromatin reticulum, but no true nucleolus. They never make amœboid movements nor ingest foreign particles, and finally they develop hæmoglobin in the cell substance, passing thus into nucleated red corpuscles. Cell division takes place with the formation of the usual karyokinetic figures. Löwit designates this method of multiplication as *divisio per fila*, in contradistinction to the method found in the leucoblasts. He states that he has never been able to find transitional forms between the two kinds of cells, though in the organs in which they occur they are found freely intermingled with each other. The leucoblasts enter the lymph stream, and eventually reach the blood as uninucleated leucocytes. These are rather small, and are devoid of the power of making amœboid movements,—a fact which was pointed out long ago by Schultze. In the blood stream, they increase in size, the nuclei become elongated and constricted, and finally fragment to form the so-called multinuclear leucocytes. He believes, then, with many others that the multinuclear leucocytes are not cells in the act of multiplication, but, on the contrary, are disintegrating; and the multinuclear stage so-called is probably followed by a complete dissolution of the cell.

In the veins coming from the blood-forming organs the uninucleated leucocytes predominate greatly in number. In the right heart the number of uninucleated forms is still relatively large, while in the left heart they become less numerous, and in the peripheral arteries they show a striking diminution. In other words, the transition from the uninucleated to the multinucleated forms takes place chiefly in the venous system during the brief interval of time required for the blood in the veins to pass from the lymphoid organs to the left heart. The erythroblasts, after the development of hæmoglobin, become nucleated red corpuscles. In the marrow of the bone all the intermediate stages may be obtained without difficulty. So in the liver and spleen of the foetus and in the spleen of the adult in some cases after severe hemorrhage similar transitional forms are found. But in the lymph glands transitional forms between erythroblasts and nucleated red corpuscles cannot be obtained. Hence he concludes that the transition in this case takes place in the lymph stream or the blood or both,

or, as a third possibility, the erythroblasts are carried to the marrow and there undergo the final changes. The nucleated red corpuscles pass into the usual red corpuscles by a loss of the nucleus. This, he thinks, occurs in the way described by Kölliker and by Neumann; that is, by disintegration and absorption within the cell. In his latest paper Löwit describes some new and rather remarkable observations upon the erythroblasts. He finds that he can obtain erythroblasts easily from the veins which bring back blood from the blood-forming organs; while in the superior vena cava they occur rarely, and in the left heart and arterial system they are entirely wanting. Though few erythroblasts are found in the superior cava and right heart, nevertheless blood from these portions of the vascular system, when treated for a number of hours with a modified Pacini's liquid, shows a considerable number of red corpuscles which contain a granular body of the shape and general appearance of a nucleus. The granules may be few or many, and in some cases they are connected by a sort of nuclear network. Löwit's description corresponds very well to the "transitional forms" of Erb which have already been mentioned. He interprets these structures as erythroblasts in which the nucleus is disappearing. Apparently, then, he believes that an erythroblast may develop its hæmoglobin and lose its nucleus by absorption while in the venous blood and during the time required for that blood to flow from the blood-forming organ to the left heart. The blood that flows from the lungs to the left heart must contain, therefore, a number of newly formed corpuscles. Nevertheless, comparisons made between the blood of the left and the right heart showed that the former contained fewer corpuscles and less hæmoglobin than the latter. Hence, during the passage of the blood through the lungs there must occur also a more or less important destruction of red corpuscles.

It has been generally believed that in the marrow the nucleated red corpuscles are not arranged in any definite way, but are mingled indiscriminately with the other elements of the marrow. Denys (14) in a recent very interesting paper states that this is not the case, at least not in the marrow of the bird. He accepts the terms erythroblast and leucoblast proposed by Löwit, and states that in sections of the marrow

which have been treated with a double stain of fuchsin and methyl green the nuclei of the erythroblasts stain green, while those of the leucoblasts stain red. Moreover, this method of staining shows that the two kinds of cells are not intermixed without order; but on the contrary they are sharply separated, the erythroblasts lying in cords or strings which are clearly marked off from the masses of leucoblasts. These cords of erythroblasts form in reality a part of the vascular system of the marrow in the following way. Between the well-defined arteries and veins of the marrow there are two capillary plexuses. One, a system of arterial capillaries comparatively few in number, is connected with the larger arteries, and is composed of long, narrow vessels with distinct, doubly-contoured walls. These open suddenly into large venous capillaries which are nearly filled with erythroblasts, and form, in fact, the cords of erythroblasts found in the marrow. The blood stream flows through these imperfectly formed vessels in a central channel which is more or less open, while the plasma probably percolates through the whole mass of erythroblastic cells. These capillaries have a very delicate endothelial wall which marks them off from the leucoblasts, and the erythroblasts filling them are so arranged that the youngest lie next to the wall and the most matured next to the central channel, where they can be floated off by the blood current. The similarity of these cords of erythroblasts or developing veins to the developing veins found in the germ layers of the embryo by Wenckebach (7) and Ziegler (8), and described and figured in the liver and posterior limb of the embryo by me, will be apparent at once. It would seem that the manner of development of the red corpuscles is the same in the adult as in the foetus. Unfortunately, Denys has not as yet shown that the same arrangement is found in the marrow of the mammal, while others positively state that no regular grouping of the blood-forming cells occurs: so that this point remains to be investigated. I shall have occasion to refer to it again. Feuerstack (17), it will be remembered, held that in the birds and other animals with nucleated red corpuscles the development of the corpuscles takes place in the blood-vessels of the marrow; but he gives no definite description of how this occurs. So, more recently, Geelmuyden describes for the marrow of the frog

what seems to be an arrangement similar to that given by Denys for the pigeon. He says that in the injected marrow of frogs the blood corpuscles do not lie free in the marrow, but are contained in definite vessels. Within the lumen of these vessels there are a great number of narrow cells which lie along the walls of the vessels, while the blood corpuscles of the circulating blood pass through the middle. Hayem (31) holds an entirely different view of the origin of the red corpuscles. Hayem, as is well known, deserves the credit of giving the first elaborate description of the blood plates. Although these elements had been mentioned, and to a certain extent studied, before his time, Hayem's investigations into their structure and meaning seem to have given the impulse to the great amount of work which has been directed to them within recent years. He attributed to the blood plates the very important function of forming the new red corpuscles. The blood plates, in fact, are in his opinion only young red corpuscles possessing the shape of the red corpuscles, — biconcave discs, — and in many cases having a greenish tint from the hæmoglobin which has begun to form in them. He speaks of the blood plates, therefore, as "hæmatoblasts." As proof for this view, he states that intermediate forms can be found between the typical blood plate and the ordinary red corpuscles, and these intermediate forms are especially numerous after severe hemorrhages when we should expect a rapid regeneration of new corpuscles. These statements, however, have not met with confirmation from the work of others. Most of those who have studied the blood plates agree in the conclusion that they do not develop into red corpuscles, however much they differ on other points. It is rather interesting that Zimmermann (32), who was one of the first to notice the blood plates, to which he gave the name of "elementary particles," also thought that they develop into red corpuscles.

Gibson (33) believes with Löwit that the spleen and the lymph glands as well as the marrow take part in the production of red corpuscles throughout extra-uterine life. To establish the fact that the spleen makes red corpuscles he removed that organ from three dogs. In two of them he was able to demonstrate a slight diminution in the number of red corpuscles, while the effect upon the number of white corpuscles was not

constant. His results were not striking, but were sufficient to convince him that the spleen has a distinct though subordinate part to play in the production of red corpuscles. As proof that the lymph glands also produce red corpuscles, he cites an experiment in which the thoracic duct was ligated for thirty-seven days before the animal was killed. Post-mortem examination showed that some of the lymph glands, especially those of the mesentery, had a reddish appearance, and contained a number of nucleated red corpuscles. Moreover, enumeration of the red corpuscles of the blood of this animal proved that a diminution of about 13 per cent. had taken place. Gibson's theoretical views of the way in which the red corpuscles are formed are as follows: In some of the colorless marrow cells the nucleus begins to increase in size, while hæmoglobin develops in the body of the cell. Later, as the hæmoglobin becomes fully formed, the cell shows a diminution in size which affects the nucleus also, so that finally one of the small typical nucleated red corpuscles is produced. Just how this becomes changed to the non-nucleated corpuscle is not stated very clearly. In one place he seems to agree with the view of Kölliker and Neumann that the nucleus fragments and is absorbed, while in other places he speaks of the nucleus becoming a blood plate. He describes the blood plates under the name of "colorless microcytes," and thinks that they are formed in part from the fragmented nuclei of the white corpuscles and in part from the fragmented nuclei of the nucleated red corpuscles. In addition to the "colorless microcytes," he describes in the blood what he calls "colored microcytes," which he believes to be the same as the "hæmatoblasts" of Hayem. These he considers to be simply fragments of red corpuscles formed in some way or other in the circulating blood. Gibson seems to be describing here the microcyte of pathological literature, small, spherical, deeply colored corpuscles very common in the blood in progressive pernicious anæmia, leukæmia, chlorosis, etc. [See Osler (24), Laache (25), Eichorst (34), *et al.*]

Obrastzow's (35) theory bears some resemblance to that of Osler already described. The nucleated red corpuscles are derived from colorless cells, which in turn are formed from free nuclei, or little spheres of nuclear matter (protileucocytes), each of which develops round itself a layer of protoplasm. The

colorless cell thus produced may change either into a nucleated red corpuscle or into an ordinary marrow cell. According to Obrastzow, the nucleated red corpuscles of most authors—*hæmatoblasts*, according to his nomenclature—possess in the living state no nucleus, the nuclear matter being diffused throughout the cell. After the death of the cell, the nuclear material becomes condensed to form a typical nucleus such as is always described for the cell. The process of condensation or separation of the nuclear matter resembles very much the coagulation of blood, nuclear substance having properties similar to though not identical with those of fibrin. The transformation of the *hæmatoblasts* to red corpuscles consists chiefly in the disappearance and absorption of the nuclear matter. Obrastzow has seen in his preparations nucleated red corpuscles, or *hæmatoblasts*, with the nuclei partially or completely extruded from the cell in the way described by Rindfleisch. He explains this, in accordance with his theory, as the result of post-mortem changes brought about by the condensation of the protoplasm after death. Arndt also believes that the nucleus of the nucleated red corpuscle does not exist in the living cell, but is formed in consequence of post-mortem changes. Indeed, he goes further than this and denies that any nucleus is present in the living red corpuscles of the lower vertebrates,—birds, reptiles, amphibia, etc. The apparent nucleus so easily seen in these cells is caused by the action of reagents or by post-mortem changes. The nucleus seen in the nucleated red corpuscles after the death of the cell consists histologically of a gelatinous ground substance containing a number of granules. He speaks of these granules as “elementary corpuscles,” and thinks that they are of the same nature as the granules found in protoplasm generally.

Afonassiew (36) concludes that red corpuscles may be regenerated in three different ways: 1. Nucleated red corpuscles multiply by division and are finally changed to non-nucleated red corpuscles. 2. The blood plates increase in size; each forms round itself an envelope of protoplasm in which *hæmoglobin* becomes developed, making a nucleated red corpuscle. This loses its nucleus by extrusion and becomes an ordinary red corpuscle. Under normal conditions this series of changes takes place only in the marrow. He seems to think that the

extruded nucleus in this case again becomes a blood plate and may enter upon a similar course of development. 3. In cases of strong anæmia one finds occasionally that certain of the red corpuscles (the poikilocytes, apparently, of the pathologist) constrict off small bits of their substance to form small red corpuscles (microcytes?) somewhat larger than the blood plates which afterwards develop into normal red corpuscles while in the circulation. Boettcher (37) contends that the red corpuscle of the blood in man and the mammalia generally is nucleated, though the nucleus under ordinary conditions is not visible. His evidence for this belief is not at all conclusive: it seems to rest chiefly upon the fact that reagents which dissolve the hæmoglobin out of the corpuscles, especially chloroform, leave behind a colorless sphere, considerably smaller than the original corpuscle, which he takes to be the nucleus. When the action of chloroform upon a red corpuscle is watched, it can be seen, he says, that the reagent dissolves off the peripheral colored portion of the corpuscle, leaving behind the colorless nucleus. Efforts to bring out this nucleus by the action of ordinary staining reagents failed except in two cases, once from the blood of a person who had died from leukæmia, and once from the blood of a tuberculous woman. It is fair to suppose that in both of these cases he was dealing with nucleated red corpuscles which had passed into the circulation.

Sappey (38) also asserts that the mammalian red corpuscle is nucleated, and that to bring out the nucleus one must treat the blood with some reagent which will make the corpuscles spherical. He recommends the following liquid: water, 500 grms.; sodium sulphate, 40 grms. Add to this solution acetic acid in the proportion of 1 to 49. Quite recently, Cuenot (39) has advanced a theory of the development of the red corpuscles which in some respects is more fanciful than any yet described. He believes that the red corpuscles are formed in the spleen, and in mammals that the whole development is carried on in this organ, while in the lower vertebrates a certain portion of the development takes place in the circulation. The spleen, according to Cuenot, contains two kinds of colorless corpuscles,—some of large size and but little refractive, which are destined to form the white corpuscles; and some of smaller size, which are very refractive, and become the nuclei of

future nucleated red corpuscles. These are not naked nuclei, but are surrounded by a very thin envelope of colorless protoplasm. The protoplasmic layer becomes enlarged, and small granules are constricted off from the nucleus, and set free in the cell. In some way these nuclear granules start the formation of hæmoglobin, either because they contain the necessary iron or because they act as a sort of hæmoglobin ferment. As the hæmoglobin develops, the granules disappear, and the nucleus becomes smaller. In the mammals the nucleus becomes entirely absorbed in the process, so that the fully formed mammalian corpuscle is non-nucleated.

If we attempt to sum up the facts with reference to the development of the red corpuscles which seem to be fairly well established, we will be obliged, as one can readily see from the foregoing review, to confine ourselves to a few fundamental points. In the first place, it is perfectly well proved that during extra-uterine life the red corpuscles are developed in the red marrow of the bones. Whether or not the spleen and the lymph glands participate in this function is not definitely determined. In the second place, it is generally admitted that the red corpuscle is first a nucleated cell, and that it loses its nucleus in the marrow or other blood-forming organ. Whether the nucleus is lost by extrusion or disappears within the cell by absorption is not settled; but the majority of writers certainly favor the latter view. In the third place, it is pretty conclusively shown that the nucleated red corpuscle is derived from a colorless cell—erythroblast, to use Löwit's term—which is formed in the marrow. The origin of this cell is the point about which, perhaps, there is least agreement. Finally, none of the recent work supports the theory that the red corpuscles are derived from the white corpuscles (leucocytes) of the circulating blood, so that this time-honored theory must be definitely abandoned.

Experimental Work.

My own work has been confined almost entirely to one mammal, the cat, partly because there was not sufficient time to make a complete series of parallel experiments and observations upon other animals, and partly because, by confining the work to a single mammal, a thorough familiarity with the

different kinds of cells was obtained, and observations made upon different individuals were capable of a closer comparison. It cannot be doubted that in its essential features, certainly, and in all probability in most of the minor details, the genesis of the blood corpuscles in the cat is the same as in man or in any of the higher mammalia.

In the course of the work I have made use of many different methods of treatment; but the methods which I have used most, and which have given me the best results, are these. When studying fresh specimens of liver blood, marrow, etc., the reagent invariably used was a 1 per cent solution of methyl green made up with 0.6 per cent solution of sodium chloride. The tissue was teased either in normal salt solution or in its own plasma, and then further teased in a drop of the methyl green. I did not use acetic acid in combination with the methyl green, as this reagent quickly dissolves out the hæmoglobin from the nucleated red corpuscles, while with the methyl green alone this does not happen unless the quantity used is too great relatively to the amount of tissue teased. The blue-green color given by the methyl green to the nucleus of the nucleated red corpuscles served to make the hæmoglobin in the cell protoplasm more distinct by contrast. The fresh tissue was examined also without the addition of any staining reagent after teasing in its own liquid, in normal salt solution, or, best of all, in blood serum which had been previously prepared from the same animal.

The marrow, spleen, and liver of the fœtus as well as the adult were studied in section, and specimens were taken from normal animals, from animals which had been bled, starved, injected, etc.

The tissue was usually hardened in a cold saturated solution of mercuric chloride according to the directions given by Gaule. Sections were cut in paraffin, and were stuck to the cover slip by the alcohol method, using 70 per cent alcohol. The sections were then stained by two or more different methods. The stains usually employed were: first, a triple stain of hæmatoxylin, eosin, and saffranin, used successively according to Gaule's method; second, alum carmine; third, Biondi's triple stain of acid fuchsin, methyl green, and orange used in mixture; fourth, the Shakespeare-Norris stain for hæmoglobin,

consisting of a mixture of borax carmine and indigo carmine. This stain was subsequently abandoned, as it was found not to work as a differential stain for hæmoglobin after mercuric chloride hardening. In several cases where sections were made of a foetal femur, with its contained marrow, the tissue was fixed in Flemming's solution, and afterwards decalcified in saturated picric acid solution. These sections treated with the indigo-carmine solution gave very beautifully the apple green stain to the hæmoglobin in the red corpuscles. Another method which I used frequently, both for the blood itself and the blood-forming tissues, is one recommended by Flemming, as follows: the fresh tissue is quickly teased upon a slide in its own liquid, and a large drop of diluted Flemming solution is dropped upon it, and the specimen then kept for twenty-four hours in the moist chamber. By that time a number of the cells have become firmly adherent to the slide, so that it can be washed in water. It is then covered with saffranin for twenty-four hours, being kept in the moist chamber. The saffranin is washed off with absolute alcohol, with or without acid, according to the depth of the stain, and the specimen treated successively with oil of cloves, xylol, and balsam. This method gave excellent results.

Development of the Red Corpuscles during Extra-uterine Life.

The importance and even the existence of the nucleated red corpuscles has been denied by some authors, as I have attempted to show in the historical review of the subject. But that these cells are found in the red marrow of the bones throughout healthy life, and that they give rise to the red corpuscles of the circulating blood, has been proved beyond any reasonable doubt, and upon the whole is as well accepted as most of the facts of physiology. What we desire, then, is a complete knowledge of the life-history of the nucleated red corpuscle, its origin, its method of growth or reproduction, and the way in which it is changed to the non-nucleated corpuscle. These corpuscles are found chiefly, if not exclusively, in the adult in the red marrow. Hence most of the work has been done upon that tissue.

Origin of the Nucleated Red Corpuscle.

Most authors agree that the nucleated red corpuscle is derived from a colorless cell existing in the marrow, but there is considerable difference of opinion as to the characteristics and origin of this cell. Löwit (29), it will be remembered, gives to it the name of erythroblast, and describes certain histological characteristics which enable him to recognize the cell wherever seen. Others derive the nucleated red corpuscles from what are known as the ordinary marrow cells, and others still, as Osler (28), describe a peculiar kind of cell in the marrow from which the nucleated red corpuscles are derived, and which correspond more or less closely to the erythroblasts of Löwit. Before speaking of my own view, it will be necessary to describe briefly the different sorts of cells found in the red marrow of the cat. In teased specimens of the marrow we meet, in the first place, with the morphological elements of the blood, the red corpuscles, white corpuscles, both uninucleated and multinucleated, and the blood plates. Of the marrow elements proper, we have, first, the nucleated red corpuscle. By this term is meant a nucleated cell colored with hæmoglobin. The size of these cells is quite variable, and they are frequently found in different stages of cell division, as described by Bizzozero (19*d*), the most common figure being the diaster. But the most marked peculiarity in the structure of the nucleated red corpuscles is found in the nucleus. In some of these cells, which for the sake of clearness I will speak of as the immature nucleated red corpuscles, the nucleus is characterized by an intra-nuclear network of chromatin, at the nodal points of which are found conspicuous granules of a similar material, which stain, however, more deeply than the reticulum. In badly preserved specimens, therefore, the nucleus seems to be composed of a number of fine or coarse granules imbedded in a clear or slightly colored matrix. The cell protoplasm of these immature forms is, as a rule, only slightly tinged with hæmoglobin, and makes a relatively thin envelope round the nucleus (see Fig. 8). Others of the nucleated red corpuscles, which may be distinguished as the mature forms, have a nucleus which shows no sign of a reticulum when stained with methyl green, hæmatoxylin, saffranin, etc. The nucleus, when stained,

shows usually, indeed, no structure whatever, but takes a deep uniform tint, as though the chromatin material were evenly diffused throughout (see Fig. 8). The nucleus of this form is generally smaller, both relatively and absolutely, than that of the immature cells; and the cell protoplasm is more deeply tinged with hæmoglobin. It is very common to find these cells with the nucleus either placed eccentrically or partially extruded, while in the immature cells no such appearance is ever seen. As the names I have chosen indicate, I consider these two forms the two extremes in the life of the nucleated red corpuscle. Intermediate stages between the extremes are, of course, of frequent occurrence; for instance, corpuscles with a nucleus which stains deeply and nearly uniformly, but shows large or small irregular clumps of a deeper staining material, like the granules of the nucleus in the younger forms, or others in which the nucleus contains smaller granules staining deeply and some indication of a reticulum between the granules; while the material between the granules and reticulum, the nuclear liquid, also takes the stain to a certain extent. The morphological difference between the two extreme types of nucleus is associated with a difference in chemical structure, as far as this can be determined by staining reagents. When sections of the marrow are treated with the triple stain, — hæmatoxylin, eosin, saffranin, — the nucleus of the immature forms takes the hæmatoxylin, while that of the mature forms stains a brilliant red with the saffranin; and the nucleus of the intermediate stages shows a combination tint of some shade of purple (see Fig. 9). The distinctness with which this difference in staining comes out depends, of course, upon the time of exposure to the different dyes. If the section has lain too long in the hæmatoxylin, all the nuclei of the preparation may be stained a dark blue or purple; while, if the exposure to the hæmatoxylin has been too short, the saffranin color predominates to the exclusion of the others. In some degree, however, the difference between the nuclei may be discovered in all cases; and when the staining has been properly regulated, it comes out with great distinctness. The time for the action of each dye varies naturally with the thickness or character of the sections; but usually a minute to a minute and a half was found to be the proper time of immersion in each of the staining reagents. It is worthy of

mention that the nucleoli of the marrow cells and giant cells, as well as the nuclei of cells during karyokinesis, when treated with the triple stain, take the saffranin in preference to the hæmatoxylin, like the nuclei of the mature nucleated red corpuscles; whereas the reticulum of the resting nucleus of most cells, unlike the nucleolus, stains most easily with the hæmatoxylin. A similar difference in the behavior of the nucleolus and the dividing nucleus has been noticed before by Steinhaus (48) for epithelial cells, and by Hodge (40) for nerve ganglion cells. With the triple stain of Biondi, the nucleus of the mature nucleated red corpuscles stains an even solid green, and in the nucleus of the immature forms the reticulum and granules at the nodal points stain a light green, while the nuclear material between the meshes of the reticulum remains unstained.

2. The next most important element of the marrow from our standpoint is a colorless cell, similar in structure to the immature form of nucleated red corpuscle, from which it differs in fact only in the absence of hæmoglobin from the cell protoplasm. The nucleus is granular without anything like a definite nucleolus. In well-preserved specimens the granules are connected by an intra-nuclear reticulum, which stains less deeply than the granules. This form of cell has been described by Osler (28), and also by Löwit (29) and others, as the progenitor of the nucleated red corpuscle. Löwit has given to the cell the name of erythroblast. It seems to me that the name is a convenient one, and I shall make use of it hereafter. At the same time, I wish to say that I do not accept Löwit's theory of the origin and permanent histological characters of these cells, which has been described in the historical review. On the contrary, my investigations have brought me to quite different conclusions, as I shall show in the proper place. Drawings of this form of cell are shown in Fig. 8.

3. The ordinary marrow cell is a large, colorless cell, with a characteristic nucleus and a faintly granular protoplasm. The nucleus is of a vesicular character, having an oval shape, a doubly contoured nuclear membrane, and one or more conspicuous nucleoli. From the nucleolus a scanty reticulum stretches out toward the peripheral membrane (see Fig 12, *a* and *b*).

4. Wandering cells. These are like 3 in structure, except that the nucleus, instead of being oval, is pulled out to an

elongated strap shape, and may be bent into a horseshoe, or may be coiled upon itself one or more times, like the leucocytes found so abundantly in the cat's blood. These cells, are, however, larger than leucocytes; and it is probable that they belong to the same class as the ordinary marrow cells (Fig. 12, *c* and *d*).

4. Some of the ordinary marrow cells have their protoplasm loaded down with coarse granules which stain readily with eosin, methyl green, etc. (Fig. 12, *e* and *t*). Sometimes these cells are very numerous: they evidently play some important part in the metabolic changes going on in the marrow. They do not appear to be confined to the marrow, since Heidentain has described what seems to be the same cell in the lymphoid tissue of the intestine, though he was unable to arrive at any satisfactory conclusions as to its function.

6. The so-called giant cells. In the red marrow of grown animals these are always of the kind described by Bizzozero as giant cells with budding nuclei to distinguish them from the multinucleated giant cell, or myeloplaque, found in developing bone, in pathological formations, etc. A more detailed description of these cells with a discussion of their functions is given in an accompanying paper.

7. Free nuclei are found sometimes in considerable numbers. In size and in the way in which they stain, they resemble exactly the nuclei of the matured nucleated red corpuscles, and there can be but little doubt that they arise from these cells.

With reference now to the origin of the nucleated red corpuscles, there seems to be little doubt that they are derived in the first place from the colorless cells (No. 2) known as erythroblasts. There has been some difference in the description of these cells as given by various observers; but there is enough agreement to justify one in believing that the same cell is meant by all, and that the erythroblast is converted to the nucleated red corpuscle by the development of hæmoglobin in the cell protoplasm. This point might be regarded as generally accepted. The real difference of opinion lies in the theories as to the derivation of the erythroblast. While Löwit (29), Denys (14), and others believe that it constitutes a distinct variety of cell found in the marrow and other blood-

forming organs, that it multiplies by indirect division, — *divisio per fila*, — and is not derived from any other element of the marrow, Osler (28) and Osbratzow (35) think that it develops from naked nuclei found in the marrow, and Foa and Salvioli believe that it is constricted off from the giant cells. The theory of Löwit is the best supported by observations and experiments, and has met with most corroboration. While I with others before and after Löwit have satisfied myself of the existence of the erythroblasts, I cannot agree with him that they are not derived from other simpler cells found in the marrow.

In sections and teased specimens of the liver of the embryo and of the marrow of the embryo and adult, I have obtained evidence to show that the erythroblasts are derived from cells of the marrow similar in structure to the ordinary marrow cells; that is, large cells with oval vesicular nucleus and a faintly granular protoplasm. Drawings intended to illustrate the way in which these cells give rise to the erythroblasts are given in Fig. 11. The marrow cells themselves have the characteristics of embryonic cells; and those from which the erythroblasts are derived are undoubtedly descendants, but little if any changed, of the original mesoblastic cells from which the marrow is formed. In the embryonic liver, as well as in the embryonic marrow, these cells are found, together with the transitional stages to the typical erythroblast. This derivation is particularly well marked in the developing blood-vessels of the liver of the young embryo. As I have already said, these vessels consist of a mass of cells destined to become red corpuscles; and some of them are typical erythroblast, while others are of the character of the marrow cells or correspond to what Löwit calls leucoblasts, and others still represent intermediate stages. None of these cells can be regarded as leucoblasts, according to the definition of Löwit, since at this time no typical leucocytes are found in the circulating blood. The embryonic cell from which the erythroblast is derived is found in the marrow of the adult as an ordinary marrow cell. In fact, the marrow cells seem to be undifferentiated cells, like the cells of the original mesoblast; and, while some may change to erythroblasts, others become loaded with coarse granules or develop into the fat cells of the yellow

marrow. Of course, there may be a difference in structure in these apparently similar cells, according to the fate which befalls them; but, if so, it is not apparent as a morphological characteristic. The ordinary marrow cell, as has been described, is characterized histologically by its vesicular nucleus, which has one or more prominent nucleoli and a scanty reticulum. In the forms intermediate between this and the erythroblast we find that the nucleoli, or nucleolar matter, becomes scattered throughout the nucleus in the form of smaller granules; while the reticulum becomes more pronounced, and unites with the granules to give the characteristic nucleus of the erythroblast. While in the latter cell, therefore, we have no distinct nucleoli, we do have a number of small granules of nucleolar material situated at the nodal points of the reticulum. This transformation from a marrow cell to an erythroblast does not take place by gradual changes going on in one cell, but makes its appearance more or less gradually in successive generations. The original marrow or embryonic cell multiplies by indirect division; and the daughter-cells, instead of having a single large nucleolus, have several smaller ones scattered throughout the nucleus and connected with its reticulum, showing thus an approximation to the structure of the erythroblast, the cells also being of a smaller size. These cells in turn multiply; and their offspring either become erythroblasts or at least resemble them more closely. One cannot say how many generations—one or more—are necessary for the change. All that can be observed is that between the large embryonic cells and the smaller erythroblasts there are found cells intermediate in size and in the structure of the nucleus; and it seems more reasonable to suppose that these changes take place after successive divisions during the re-formation of the nucleus from the chromatin filaments rather than from a process of condensation and alteration going on in each cell. Denys (14) has found in the marrow of birds that the erythroblasts are separated from the other elements of the marrow, and lie in cords, which are in reality a part of the vascular system of the marrow. I have described a similar arrangement in the liver of the young embryo cat. But if such an arrangement of the erythroblasts exists in the marrow of the cat, it is certainly very much obscured, as repeated examina-

tions of sections of the marrows of cats of all ages has not revealed a separation of this character. On the contrary, the erythroblasts seem to be scattered among the other elements of the marrow without any apparent regularity. It is possible that careful injection of the marrow will throw more light upon the subject. On *à priori* ground, I should think that in the mammalian marrow there must be some such arrangement as that described for the bird and the embryo, as it would furnish the simplest explanation of the way in which the newly formed red corpuscles develop and gain entrance into the circulation, and would prove that the process of formation in the adult and foetus and among the chief classes of vertebrates is essentially the same. The embryonic cells from which the erythroblasts are formed must also, of course, lie in the unformed vessels with the erythroblasts, as is the case in the embryo.

Growth and Reproduction of the Nucleated Red Corpuscles.

Since the observations of Bizzozero (19) it has been known that the nucleated red corpuscles multiply by indirect division (karyokinesis) like most of the other cells of the body. Though his observations have not been disputed, other writers have described different methods of growth, some of which have been mentioned already. Foa and Salvioli (13) believe that the nucleated red corpuscles are recruited continually from the giant cells, Löwit (29) that they are developed from the erythroblasts, and Malassez (27), Osler (28), and others take a similar view. None of them, except Bizzozero, seem to lay much stress upon the independent reproduction of the nucleated red corpuscles themselves. It is quite easy to show, nevertheless, that Bizzozero's observations are perfectly correct, and that not only the erythroblasts, but the nucleated red corpuscles also, multiply by indirect division. Simple examination of teased specimens of the marrow, especially of kittens which have been bled severely, gives usually a number of corpuscles undergoing division, such as are shown in Figs. 5 and 10. Specimens teased in methyl green solution show sometimes a portion of the spindle, as indicated in the figure; but the chromatin filaments are not well preserved. The reagent seems to swell the filaments into a mass, but, in spite of this, it is

not difficult to recognize the chief stages of karyokinetic division. When the marrow is preserved in Flemming's solution, and the sections are stained in saffranin, the nuclear figures are very well preserved, and undoubted nucleated red corpuscles, showing the skein, monaster and diaster, can be obtained without trouble, as shown in Fig. 10. Nucleated red corpuscles with two nuclei and the cell partially constricted between, — that is, the last step in the process of division, — are especially common. We must admit, then, that the nucleated red corpuscles have the power of independent multiplication. But this power of reproduction is not unlimited; and this, it seems to me, is an important fact which has hitherto been overlooked. It is not difficult to determine when the cell has lost its power of reproduction: it is indicated plainly by the appearance of the nucleus. The changes in the structure of the nucleus of the nucleated red corpuscle have been described already in detail, especially the two extremes designated as the mature and immature form of the nucleus. The immature nucleated red corpuscles have a nucleus like that of the erythroblast, preserving a definite reticulum, and, like the erythroblast, it is capable of karyokinetic division. But the offspring or daughter-cells of this form have nuclei belonging to the intermediate class, in which the reticulum is less marked, and the whole nucleus shows a tendency to diffuse staining. These cells are very common in the marrow, and it is probable that they also are capable of multiplication. But sooner or later the offspring of these cells show nuclei with no reticulum at all, and staining diffusely and deeply with the different dyes. This is the mature form, and is further characterized by the deeper color of the hæmoglobin in the cell substance. This cell is now ready to lose its nucleus, and become an ordinary red corpuscle; and, as far as I can determine, nucleated red corpuscles which have reached this stage are incapable of any further multiplication. The mature corpuscles are usually smaller than the immature forms, as the successive offspring show a gradual diminution in size both of the nucleus and the cell substance. It is impossible to say how many generations intervene between the youngest nucleated red corpuscle, in which hæmoglobin has just appeared, and the mature form, with its peculiar nucleus and greater hæmoglobin contents.

The number, of course, may not be constant, at least not for different conditions of life. All that one can actually observe, and this point I wish to emphasize, is that the cells which I have described as the mature and immature forms of the nucleated red corpuscle really exist in the marrow at all times, that the latter undoubtedly multiply by karyokinesis, and that the former bear every indication of being nearer the condition of the non-nucleated red corpuscle, both in size and depth of color, and in the fact that they are no longer capable of reproduction. The theory which I have suggested offers a simple explanation of these phenomena. One other hypothesis which might be suggested, and which has in fact been proposed, is that the nucleated red corpuscle, after it has been formed from the erythroblast by the development of hæmoglobin, begins to undergo a process of condensation which results in making both the cell and the nucleus smaller. But this theory does not take into consideration the fact that what I have called the younger forms of the nucleated red corpuscle are without doubt capable of active multiplication, and that the offspring seem to show in general a diminution in size and a definite change in the character of the nucleus.

The Transformation of the Nucleated Red Corpuscle to the Red Corpuscle of the Blood.

The essential factor in the transformation is the loss of the nucleus. After it was known that in the foetus the nucleated red corpuscle loses its nucleus and changes to the non-nucleated form, Kölliker (3) proposed the theory that the nucleus is destroyed by absorption within the cell. The absorption may be preceded by a fragmentation of the nucleus more or less complete, such as one often sees in examining the blood of a young embryo. Kölliker does not seem to have given any microscopic proof for his view other than the partial disintegration of the nucleus. Neumann (5*b*), after he had clearly shown that the nucleated red corpuscle exists also in post-natal life as the precursor of the non-nucleated form, adopted the theory of Kölliker to explain the disappearance of the nucleus. He was able to follow the process best in the human foetus (five months), and describes the nucleus as becoming smaller, more homogeneous, and finally notched or indented. In addition, he describes red

corpuscles with only one or two small granules of nuclear matter, which he takes to represent the last step in the disappearance. There is very little satisfactory proof, then, for the theory, since no one, of course, has been able to follow the process through all its changes, and the appearances described above might easily be explained in other ways. Nevertheless, the theory has been generally adopted by those who believe in the nucleated red corpuscle and its functions. Malassez, of course, upon his theory of budding, is not obliged to explain the loss of the nucleus, nor are those who believe in an endogenous formation of the red corpuscles; but, outside of these theories, which cannot be said to have a strong support at present, the general belief among histologists is that the nucleated red corpuscle loses its nucleus by absorption in the way described by Kölliker and Neumann. There seems to be, indeed, only one other alternative: if the nucleated red corpuscle changes to the non-nucleated form, the nucleus either disappears by absorption within the cell or by extrusion from the cell. This latter view has been seriously supported only by Rindfleisch (26). As I have stated in the historical review, Rindfleisch believes that the nucleus escapes from the nucleated red corpuscle surrounded by a small layer of colorless protoplasm, and leaves behind a bell-shaped corpuscle which eventually becomes a biconcave disc. He figures corpuscles in which the nucleus was seen in the act of escaping from the cell. Others have seen similar examples of extruding nuclei, but have concluded that it was an accidental and not a normal phenomenon. The chief result of my own work has been to obtain what seems to me indisputable evidence that the extrusion of the nucleus is the normal method by which the nucleated red corpuscle loses its nucleus and passes into the non-nucleated form. Unlike Rindfleisch, I have never been able to discover with the highest objectives (Zeiss Hom. im. $\frac{1}{8}$ and apochromatic im.) that the escaping nucleus has an envelope of protoplasm round it. On the contrary, it goes out of the corpuscle entirely naked, and can be found as a free nucleus in sections and teased specimens of the marrow, and also in the embryonic liver, as has been previously described by Neumann (see Fig. 2). In many cases in the marrow, and especially in the foetal liver, I have seen the homogeneous nucleus partially

segmented or notched in the way described by Kolliker (?) and Neumann, and interpreted by them as an indication that the process of absorption had begun. Nevertheless, I have seen nuclei of this character already partially extruded from the cell, showing that the partial fragmentation of the nucleus is not conclusive proof that it is in process of absorption. To show that the escape of the nucleus is a normal and constant phenomenon we have the following facts:

In specimens of the marrow of kittens and adult cats, especially after repeated bleedings, and also in the blood-forming organs of the embryo when teased out in their own serum and stained with methyl green, one can easily find very many examples of nucleated red corpuscles in the act of losing their nuclei. In some animals the number of examples is striking—a dozen or more may be seen in a single specimen; while at other times, especially in unbled animals, it may be difficult to find a single example. But in bled animals, especially bled kittens, in which it is fair to suppose that the process of blood formation is greatly accelerated, no difficulty will be found in obtaining a number of examples showing all the steps in the act of extrusion, from the time when the nucleus has only an eccentric position up to the period when it lies completely outside the cell, as shown in Fig. 2. The frequency with which this phenomenon occurs, especially when the production of red corpuscles is increased, requires that it should be explained. Now it must be a normal occurrence, or else it comes from the action of the reagents, or possibly it is the result of post-mortem changes taking place in the cell after removal from its normal environments.

There are a number of facts which may be adduced to show that the phenomenon is not an accidental or post-mortem change, but a normal occurrence. In the first place, most of the specimens were obtained from pieces of the marrow (or liver in the embryo) which were taken as quickly as possible from the animal after killing, and treated with methyl green, so that only a few minutes intervened between the death of the animal and the action of the methyl green. This reagent, as is well known, is an excellent fixative. It preserves fairly well the nuclear figures of karyokinesis, and fixes the blood plates quite as well as osmic acid. It is not likely, then, that such a re-

agent would cause in one of the cells of the marrow an expulsion of the entire nucleus, and in others preserve the delicate karyokinetic figures; and, on the other hand, the fact that the marrow was submitted to the action of the reagent so quickly after the death of the animal, probably before the death of the marrow cells, precludes the possibility of post-mortem changes of the nature required to expel the nucleus from a cell. So in several cases, both in the adult and the kitten, after severe bleeding, and also in the foetus, I have found examples of extruding nuclei in the circulating blood. In these cases, the drop of blood was taken from the living animal and mixed at once with the methyl green, so that there was no opportunity for post-mortem changes (see Fig. 2). Moreover, I have obtained cases of extrusion frequently in sections of marrow which had been taken from the animal as quickly as possible after bleeding, and hardened in mercuric chloride. Here, again, we have an excellent fixative quickly applied, which ought to have prevented post-mortem changes on the one hand, and on the other should not have acted with such violence upon one of the kinds of cells found in the marrow as to force out the nucleus. To adopt either one of these hypotheses to explain the extrusion is not permissible in the light of our knowledge of the action of this reagent on cells in general.

In the second place, all the red corpuscles which I have seen with the nuclei extruding belong to the class of mature nucleated red corpuscles. Never have I seen a nucleus extruding from a nucleated red corpuscle which showed a nuclear network. This indicates that the escape of the nucleus is not owing to any accidental or post-mortem changes, since there is no reason under such conditions why all kinds of nucleated red corpuscles should not have been affected in the same way. It shows, also, that the extrusion of the nucleus is the normal end to the life history of the nucleated red corpuscle, since it is found only among those which seemed to have reached full maturity and are prepared, as far as size, color, etc., are concerned, to become ordinary red corpuscles. It seems to me that this fact is a very important one in its bearing upon the question under discussion, and, so far as I know, it has not been noticed before. I have been impressed with this pecu-

liarity of the extruding nucleus, not only from the study of teased specimens stained in methyl green, but also from an examination of sections of marrow stained with hæmatoxylin, eosin, and saffranin. It is not difficult to find in these sections a nucleus in the act of extruding, and in all cases such nuclei belonged to the mature nucleated red corpuscles as shown by the fact that they stain with saffranin in preference to the hæmatoxylin in the way that I have described. Osler (28), who has figured and described the extruding nuclei, but does not think they occur normally in the living tissue, states that they are more abundant in the marrow twenty-four hours after death than in the fresh cadaver. This may well be, even if the phenomenon is a normal occurrence, since the marrow cells probably survive some hours after somatic death, and the mature nucleated corpuscles may lose their nuclei partially or completely as in life, and the stoppage of the circulation would lead to an accumulation of such examples in the marrow. However, in the cat, at least, under the conditions mentioned, they can be found in abundance immediately after death. Whether or not with this animal the number is increased twenty-four hours after death I have never determined. The presence of granules within a newly formed red corpuscle has been taken as a proof that the nucleus is absorbed within the cell, the granules being looked upon as remnants of a former nucleus. The existence of such cells cannot be questioned; but, taken alone, they cannot be considered as strong proof for the theory of absorption nor as any objection to the theory of extrusion; for I have in a number of cases found red corpuscles containing these granules in which, nevertheless, the nucleus was in the act of extruding, as shown in Fig. 2. The granules in such cases evidently did not mean that the nucleus had been absorbed. Erb (4), it will be remembered, described such corpuscles in the circulating blood; they form his transitional stage between the white and red corpuscle. Löwit (29*d*) has newly discovered them, especially in the blood of certain veins after treatment with a modified Pacini's liquid, and has laid great stress upon them as transitional forms between the erythroblasts and red corpuscles. Foa (41) also has recently described granulations of this character as part of the normal structure of every red corpuscle and easily brought out

by appropriate treatment with methyl blue and chromic acid. I have met with corpuscles containing granulations very frequently, particularly in the blood-forming organs. In sections or teased specimens of the blood-forming organs, the newly formed red corpuscles are often characterized by the ease with which they lose their hæmoglobin. Under such conditions the granulations come out very distinctly. Sometimes the granules — which stain, by the way, like nuclear chromatin — are so arranged as to represent the outline of the nucleus, and I have obtained such cells in which the nucleus at the same time was fixed in the act of extrusion (see Fig. 7). It is an interesting fact with reference to the corpuscles containing granules that they are usually newly formed corpuscles, and on that account occur most abundantly in the foetal blood or in the blood-forming organ (marrow) of the adult. There is no evidence to show that the granules are the last remaining fragments of an absorbed nucleus. On the contrary, all that we know about them is opposed to such a view. They must be looked upon, it seems to me, as bits of the nuclear chromatin (membrane) left behind when the nucleus leaves the cell. What their fate is, whether finally absorbed or whether they last throughout the life of the corpuscle, is not known.

In this connection I may refer to a curious phenomenon which has come under my notice and upon which I am now working. On one occasion, after bleeding a medium-sized cat very severely (a loss of 90 cc. of blood), it was found upon examining the blood twenty-four hours afterward that the majority of the corpuscles in the animal contained a single good-sized piece of nuclear matter, too large to be called a granule, but having the shape and appearance of a large nucleolus. This fragment stained readily with methyl green just like the nucleus: it could be seen also in the unstained corpuscles as a refractive particle (see Fig. 4). I cannot recall ever having seen anything corresponding to this described, except, perhaps, the first stage of the malarial germ as pictured by Marchiafava, with which, indeed, the appearance seen by me seemed to be identical. Closer examination of the corpuscles showed that the fragment of nuclear matter, as I shall call it, always lay imbedded in the periphery of the spherical corpuscle after treatment with the methyl green. When care was taken to

make the corpuscle rotate in the liquid, I found no exceptions to this position of the fragment. A remarkable thing about the phenomenon was its persistence. Even two weeks after bleeding, a drop of the blood taken from the ear showed a number of these corpuscles. I was successful afterwards in getting the same result from other cats, though I had many failures. The necessary condition seems to be that the animal should be bled quickly and severely. At first, I supposed that the objects in question were simply large granules floating in the blood which had adhered to the corpuscles; but I was soon convinced that this was not the case. The fragments could not be detached from the corpuscles either by shaking or by the addition of water, acetic acid, and other reagents, which dissolve out the hæmoglobin from the corpuscles. Moreover, a number of corpuscles were without the fragments, and in normal cats no such appearance could be obtained. The only satisfactory explanation of the phenomenon which has occurred to me is that the fragment is a bit of the nucleus left adhering to the corpuscle at the time that the nucleus escaped. Under the conditions necessary for the appearance of the phenomenon, we may suppose that the process of production of new red corpuscles was vastly accelerated, and that therefore the extrusion of the nucleus was not as perfect as under normal conditions. The portion remaining in the corpuscle is not absorbed at all, but probably remains with the corpuscle up to the time of its dissolution. Whether or not my view as to the origin of the fragment is correct, there can be no doubt that it is not absorbed in the corpuscle while in the blood, but remains with it up to the time of its destruction. At the suggestion of Dr. Bowditch, I had hoped to use the phenomenon to measure the average length of life of the red corpuscle of circulating blood, but have hitherto met with certain difficulties which I hope soon to overcome.

After I was convinced from a study of teased specimens and sections that the nucleated red corpuscle loses its nucleus by extrusion, it seemed to me that it might be possible to watch the process taking place in the living cell. The experiments that I made for this purpose were not very numerous, for reasons that will be given below; but they were successful in a measure, at least. The method employed was to use the

marrow of very young kittens, about a week old, which had been bled rather severely from the jugular vein some twenty-four hours previously so as to increase the processes of blood formation. The marrow was teased out quickly in an indifferent solution of some kind upon a slide, the edges of the cover slip were sealed with paraffin, and the slide was kept at a temperature of 37–38° C., by means of a warm stage. Various indifferent solutions were tried, such as normal salt solution, amniotic liquid, aqueous humor, and blood serum; but successful experiments were obtained only when the serum of the same animal was used as the teasing liquid. The other liquids were given only one or two trials; but as far as the experiments went, they indicated that even such liquids as normal salt solution and amniotic liquid are sufficiently abnormal to cause a suspension of the living activities of the nucleated red corpuscles. Two experiments were made with the animal's own serum as the teasing liquid. In the first I saw two cases of extrusion, in the second only one, in which I was able to follow the process in part at least. In picking out the corpuscle to be observed I found it was necessary to choose one in which the nucleus already showed signs of extrusion, for otherwise it would be impossible except by accident to select a cell which had reached the proper stage. It was not difficult to find a number of corpuscles with the nucleus beginning to extrude. Many of them showed no further change, though watched for some time; but in three cases I was able to follow the last stages of extrusion until the nucleus lay completely outside of the cell. Sketches were made of one of these successful cases, though unfortunately it was the most incomplete of the three. The drawings are given in Fig. 2. The experiments were discontinued because of the improbability of obtaining a cell in which the process could be watched from the beginning to the end. The results, as far as they went, were still further proof to me that the extrusion of the nucleus is a normal phenomenon, since it was obtained only when the conditions were most favorable for preserving the life of the cell. I have spoken of the escape of the nucleus as an extrusion, but it is quite possible that migration would be a more accurate term. I was not able to convince myself that the escaping nucleus in the living cell showed definite amœboid movements,

though the sketches made (see Fig.) seem to indicate that such movements occur. The figure shows, indeed, that the corpuscle as well as the nucleus undergoes changes in shape; but this was caused in part at least by the rolling of the cell so as to present different surfaces in successive drawings. *A priori*, it seems much more likely that the extrusion should result from some active movement on the part of the nucleus rather than from contractile changes in the cell substance. For it seems to be generally admitted now that in certain cells — lymph cells especially (Arnold) — not only movements of the nucleus may take place, but movements of the granules and filaments in the nucleus. After the escape of the nucleus, the spherical red corpuscle eventually becomes a biconcave disc. I have not attempted to follow this change, though I feel convinced that the bell shape which Rindfleisch ascribes to the corpuscles which have just lost their nuclei is a mistake. The red corpuscles even of the circulation, as is well known, frequently take this shape when treated with reagents of any kind, or even when examined without the addition of any liquid. It seems to me very natural to suppose that the biconcavity of the mammalian corpuscle is directly caused by the loss of the nucleus from its interior. Certainly as long as the corpuscles in the foetus and the adult retain their nuclei, they remain more or less spherical, and after they lose their nuclei they become biconcave. The mechanical conditions of the circulation undoubtedly have some influence upon this change, but the initial cause lies apparently in the migration of the nuclear mass from the middle of the cell, so that the viscous material of the corpuscle is permitted to sink in. The biconcavity is of course a decided physiological advantage, as the absorptive surface is thereby considerably increased, so that upon the doctrine of natural selection, one can readily understand why such a variation should have become permanently established. Among the Camellidæ, it is true, we have biconvex non-nucleated corpuscles. So far as I know, no one has investigated the hæmatopoietic function in these animals, but it is possible that small spherical erythroblasts are not formed in them as in the other mammalia.

If we grant that the nucleated red corpuscle loses its nucleus by extrusion when it passes to the non-nucleated form, then

we are in a position to explain the budding corpuscles of Malassez. In several instances, when examining the marrow, I have met with appearances which seemed to justify Malassez's theory. Nucleated red corpuscles were seen with one or more non-nucleated corpuscles apparently budding out from them. Sketches of such cells are given in Fig. 3. They seem to me, indeed, to be better examples, as far as the drawings go, of the process of budding than those figured in Malassez's (27) own paper. I cannot say that these examples of budding are common; on the contrary, I obtained them clearly only in two cases, in both of which the notes of the experiments record that the animal had been bled so severely that it did not make a good recovery, but remained weak and anæmic; and it is possible that this is sufficient to explain their occurrence. I was at first inclined to believe that we must admit that, under certain conditions at least, new red corpuscles may be produced by budding in the way described by Malassez. But a simpler explanation of these forms suggested itself. What seem to be examples of budding are most probably cases of multiplication of nucleated red corpuscles by division, in which the process was not carried out to the complete separation of the newly formed corpuscles, though from one or more of the new cells formed the nucleus has escaped, leaving the non-nucleated corpuscle as an apparent bud on its sister-cell. As evidence for this explanation, one may find in the apparent buds granules of nuclear matter staining blue with the methyl green, such as I have described as occurring sometimes in the newly formed red corpuscle after the extrusion of its nucleus. Moreover, one frequently meets with two, three, or more mature nucleated red corpuscles joined in a cluster or chain as the result of recent division, and such as would produce apparent examples of budding if one or more of the cells lost their nuclei. This would be more likely to happen, of course, in animals in which too severe a bleeding had impaired the processes of cell development in the marrow as in the other tissues of the body. The explanation that I have adopted seems to me to be preferable to supposing that in the marrow new blood corpuscles are formed from the same cells by two entirely different methods of reproduction.

Fate of the Extruded Nucleus.

If the nucleus of the nucleated red corpuscle is extruded, the next point to be determined is what becomes of it. Naked nuclei, similar in all respects to the nuclei of the mature nucleated red corpuscles, can be found easily in the marrow, where, indeed, several observers have called attention to them, and also in the foetal liver at the time of its hæmatopoietic activity, where they have been noticed before by one writer, at least, — Neuman (5*d*), — who has described them very carefully and attributed to them some function in connection with the production of new corpuscles. It is fair to assume that the free nuclei are turned out into the blood stream along with the new red corpuscles. In that case, one of two fates awaits them. Either they persist as a morphological element of the blood, or they are dissolved in the blood plasma. Upon the first hypothesis, we can only suppose that the free nuclei become the blood plates, as no other element of the blood resembles them in size or structure. This theory has, in fact, been proposed by Gibson, though as far as I can see, he gives no proofs in its favor. I was also at first impressed with this idea; but the only experiment which suggested itself to me to test the hypothesis gave me unfavorable results. The nuclei of mature nucleated red corpuscles, when treated with the triple stain of hæmatoxylin, eosin, and saffranin, show a preference for the saffranin, while other nuclei take the hæmatoxylin. If the blood plates are derived from these nuclei, they ought to show something of the same behavior toward the triple stain. On the contrary, specimens of blood plates treated with the triple stain always take the hæmatoxylin, though they do not stain deeply. The method of preparing and staining the blood plates was as follows. A drop of blood was placed upon a slide, a cover slip was dropped upon it, and moved round once or twice. The slip was then taken off, and by this time a number of blood plates had adhered to its under side. It was next immersed in Hayem's liquid, to fix the blood plates and wash off the excess of blood plasma, and was then hardened like a piece of marrow in mercuric chloride, followed by alcohol, and afterwards stained. I obtained in this way good specimens of blood plates, somewhat deformed, of course, in

consequence of the time which elapsed before getting the slip into Hayem's liquid. The method also gave beautiful permanent specimens of fibrin reticulum and of red corpuscles, which retained their normal shape and stained deeply with eosin. The negative result of this experiment, together with certain other facts which will be given later in speaking of the blood plates, convinced me that there is no connection between the blood plates and the nuclei of the mature nucleated red corpuscles. There remains, then, only the theory that the liberated nuclei are dissolved in the blood plasma, and go to form in all probability one of the proteids of the blood.

It is, perhaps, unwise to speculate further upon the fate of the dissolved nucleus without some experimental basis to reason upon. However, my idea is that the free nuclei are dissolved in the blood plasma while still in the blood-forming organ. I have seen appearances in the marrow in sections which may represent this process of dissolution; that is, one meets occasionally with what seem to be globules of varying size from tiny drops to spheres larger than a white corpuscle which, like the free nuclei, stain deeply with saffranin, though of a different tint. Usually these are found in clusters of different sizes, and possibly they represent the free nuclei, undergoing changes preparatory to solution, though I have not found intermediate stages. These globules are evidently not a fat of any sort, as one might suppose from their general appearance, since otherwise they would have been dissolved during the process of imbedding. With reference to the material produced by the nuclei after solution, there seemed to me certain reasons for believing that the fibrinogen of the plasma is the product formed. Influenced chiefly by this idea, I asked Mr. Dreyer of the Johns Hopkins University, and formerly assistant in physiology, to investigate the changes in the blood plasma caused by severe bleeding. His results, which are very interesting in a number of ways, have not yet been published. It may be said, however, that with reference to the fibrinogen, he found that its percentage in the plasma was always increased, sometimes nearly as much as 100 per cent., over what it had been in the same animal before bleeding, the analysis in all cases having been made twenty-four hours after the bleeding. This striking increase in the fibrinogen is more remarkable because

at the same time there was usually a diminution in the total proteids of the blood. As far as it goes, this result is in accord with the hypothesis that the fibrinogen is formed from the liberated nuclei of the nucleated red corpuscles. I have in progress other experiments for the purpose of further testing the hypothesis.

The Hæmatopoietic Function of the Spleen.

All the facts bearing upon this question have already been stated in various parts of this paper. It may be convenient, however, to bring them together in the form of a brief statement of the different views held. It is well known and universally admitted that for a certain period during embryonic life, the spleen takes part in the formation of red corpuscles, as is shown by the fact that numerous nucleated red corpuscles, some of them in the act of multiplication, may be found in it. Shortly after birth, the spleen no longer contains nucleated red corpuscles and for this reason the majority of writers who believe that these cells are the predecessors of the ordinary red corpuscles, have concluded that under normal conditions the spleen during extra-uterine life takes no further part in the production of new red corpuscles. This function is relegated entirely to the red marrow. On the other side, a number of investigators, while admitting the absence of nucleated red corpuscles from the spleen under ordinary conditions, have nevertheless classed it with the lymph glands under the head of the hæmatopoietic organs, because they hold that the colorless corpuscles from which the nucleated red corpuscles are formed are produced in this organ. The most elaborate form of this theory is found in the works of Löwit (29) upon the origin of the erythroblasts, an account of which is given in the historical review. For my own part, I have not been able to convince myself that erythroblasts are continually forming in the spleen or lymph glands, as I have not been able to get any intermediate stages between them and the nucleated red corpuscles, and therefore take sides with those who think that the red marrow is the only organ as yet discovered, in which new red corpuscles are produced during post-natal life. This statement applies, however, only to the spleen under ordinary conditions of life. Bizzozero (19)

was the first to discover that in a number of animals, after severe and repeated bleedings, the spleen again might contain nucleated red corpuscles showing signs of active multiplication. This was denied by Neumann, who held that after such an operation, the nucleated red corpuscles found in the spleen were not more numerous than those present in the circulating blood. But Bizzozero's observations have met with confirmation at the hand of others, — Gibson (33), Foa (22), *et al.*; and I also in several cases have been able to show without any difficulty that in the cat, after severe and repeated bleedings, and in some cases after a single strong hemorrhage, nucleated red corpuscles can be found in the spleen with every indication that they are multiplying there. The balance of evidence is strongly in favor of this power of the spleen to resume its embryonic function when the demand for new red corpuscles is very urgent. In what way severe anæmia stimulates the spleen to a renewal of its hæmatopoietic activity is not known. It is very interesting in this connection to find that, when the spleen of the adult is partially excised, it is regenerated, and during the regeneration not only nucleated red corpuscles, but giant cells are found just as in the developing spleen of the embryo (Foa [42], Tizzoni [43], Griffini [44]). It may be that in the adult spleen a number of undifferentiated or erythroblastic cells are contained which become aroused to activity in consequence of severe anæmia, for the same reason, whatever it may be, that the cells of the marrow are stimulated to increased growth and multiplication by the same conditions.

Life-History of the White Corpuscles and Blood Plates.

It is quite generally agreed that the origin of the white corpuscles of the blood is to be found in the lymph leucocytes, or lymphocytes, to borrow a convenient term, which in turn are formed in the lymphoid tissues of the body, and especially in the so-called compound lymphatic glands. The lymphocytes are characterized by a vesicular nucleus, usually with a nucleolus and a scanty reticulum, and by a very small protoplasmic envelope. In the blood we meet with two chief varieties of leucocytes, — uninucleated and multinucleated. The uninucleated forms do not all have the same structure: some of them

resemble exactly the lymphocytes, and may be regarded as lymphocytes newly arrived in the circulation and as yet unchanged in structure (Erb, Löwit). These are characterized physiologically, as was pointed out some years ago by Schultze (45), by not possessing the power of making amœboid movements. A second form of uninucleated leucocyte is characterized by its large, finely granular, protoplasmic envelope. This form is amœboid, and it seems most reasonable to suppose that it is derived from the first form, or lymphocyte, since this latter cell is the only or chief form in which the leucocytes of the lymph enter the blood. The first variety of uninucleated leucocyte passes into the second in consequence of a growth in the cell protoplasm while in the blood current, the protoplasm meanwhile acquiring the power of contractility. A third variety of uninucleated leucocyte, and what seems to represent a third stage of development, is like the last, except that the nucleus is no longer oval or spherical, but is drawn out to an elongated strap shape, and may take either a horse-shoe form or may be more or less coiled into a spiral. This form of cell is especially abundant in the cat's blood, and possesses the most active amœboid properties. The origin and meaning of the multinucleated forms has been for some time a subject of dispute among histologists. Formerly it was generally thought that they represented cells in process of multiplication by direct division; and this view is still warmly supported by Arnold and others. The normal fate of the multinucleated cell, according to this view, is to divide into a number of cells corresponding to the number of nuclei. Others, and especially Löwit (29), have urged that the multinucleated forms are cells on the way to disintegration, and the so-called nuclei are made simply by the fragmentation of the nucleus of a uninucleated leucocyte, and represent the first step in the process of destruction. As far as my observations go, they support Löwit's view. I have never seen any indication of the multinucleated cells segmenting to form new cells. On the contrary, there is every reason to believe that they are undergoing a course of retrograde changes, the normal termination of which will be the disintegration and dissolution of the cell. With reference to the derivation of the multinucleated forms from the uninucleated by fragmentation of the nucleus, I have been able

to find all intermediate stages in the process as shown in Fig. 16. They are derived always from the third variety or third stage in the life of the uninucleated leucocyte, the elongated nucleus breaking up into the smaller fragments; and it is not difficult to find cells such as are shown in the figure in which the fragmentation is going on. According to this view, the different varieties of leucocytes found in the blood are in reality different stages in the life-history of the white corpuscle, and pass one into the other. To complete the life-history, one other stage must be described,—that of the disintegration of the multinucleated form. A close examination of the multinucleated cells, especially when in the act of disintegrating, has impressed me with the belief that the fragmented nuclei persist for a certain time in the circulation as the blood plates, though doubtless the blood plates also, sooner or later, go into solution.

This view of the origin of the blood plates is not new. Gibson (33) supports it, and gives some evidence in its favor; and Hlava (47) especially has given a number of arguments—none of which, however, are very conclusive—to prove this derivation. One is led, at first, to such a theory by noticing the very striking resemblance between well-preserved blood-plates and the fragmented nuclei as far as size, shape, and general appearance are concerned. This resemblance is still further increased when the blood plates are examined in the blood of an animal which has been repeatedly bled. Under such conditions, one gets, or may get, blood plates which have one or more granules within them staining more deeply than the rest of the plate, and resembling very closely the chromatin granules found in the fragmented nuclei of the leucocytes, as shown in Fig. 6. Something similar to this seems to have been obtained by Afonassiew. We may suppose in this case that the increased activity in the processes going on in the blood in connection with the regeneration, not only of its formed elements, but of its characteristic proteids, have led to a more rapid breaking down of the leucocytes, and that some of the fragmented nuclei are liberated as blood plates before reaching the usual degree of maturity. There is, moreover, a very close similarity in the way in which the fragmented nuclei and the blood plates stain. As far as I have been able to test them,

they stain alike, except that the blood plates take the stain more feebly. In the case already mentioned, in which the preserved blood was treated with a differential stain, successive staining in hæmatoxylin, eosin, and saffranin, the blood plates, like the nuclei of the leucocytes, took the hæmatoxylin. The same is true of methyl violet (Gibson) and methyl green. If this view of the life-history of the leucocytes of the blood is correct, it seems probable that they play an important part in the formation of the blood proteids. The young lymphocytes increase in size by the formation of new protoplasm; and in the end this again passes into solution in the plasma. Schmidt long ago stated that the paraglobulin of the blood is derived from disintegrated leucocytes. In fact, if I understand him correctly, he believes that the paraglobulin is all formed in this way after the blood is shed. Later investigations of the serum and plasma have shown that this latter statement is not correct, though there is apparently an increase in the amount of paraglobulin in the serum over that in the plasma. Still, it may be considered probable that the paraglobulin of the blood is derived wholly from the breaking down of the leucocytes, and that the constant supply of paraglobulin in the blood is derived from the continual disintegration of the multinucleated leucocytes. The fibrinogen, on the other hand, is possibly derived from the liberated and dissolved nuclei of the mature nucleated red corpuscles, and perhaps of the blood plates also, if they, too, represent nuclear material. We know little or nothing at present of the genesis and relationship of the blood proteids or of the nutritive value and significance of each. The fact that their percentage amounts in the plasma remain practically constant under many different conditions of nutrition indicates that they are regenerated continually in proportion as they are used up; but how this happens is one of the darkest as well as one of the most interesting points in the physiology of the blood. It seems to me that the question must be studied, in part at least, upon the hypothesis of their derivation from the formed elements of the blood in the manner here suggested, somewhat as we look upon the ground substance, or matrix, of the connective tissues as having its origin from the cellular elements.

SUMMARY.

The chief conclusions to which the investigation has led may be briefly summarized in the order in which they are presented in the paper as follows:—

1. In the very young embryo two forms of red corpuscles are found,—one large, oval, and always nucleated, resembling the corpuscles of the lower vertebrates, and one small, biconcave, circular in outline, and found both nucleated and non-nucleated. The latter are the true mammalian corpuscles; the former represent possibly ancestral corpuscles. The true mammalian corpuscles lose their nuclei by extrusion.

2. In the first part of embryonic life new red corpuscles are produced in the liver from groups of mesoblastic cells outlining the position of future blood-vessels (veins). The central cells of these cords become red corpuscles, while the peripheral ones form the walls of the veins. Similar developing blood-vessels are found in the embryonic muscular tissue of the posterior limb. It is probable that new red corpuscles are formed in all parts of the body where blood-vessels are being developed.

3. In the second half of the embryonic life red corpuscles are formed in the liver, the spleen, and the marrow of the bones, the function being most active first in the liver, then in the spleen, and finally in the red marrow. In the cat the liver and spleen lose this function three or four weeks after birth, and henceforward the red marrow alone produces new red corpuscles.

4. The white corpuscles (leucocytes) and blood plates do not occur in the circulating blood of young embryos, but make their appearance in later embryonic life. In the human foetus of five months both are present.

5. In the healthy animal during extra-uterine life the red corpuscles are produced only in the red marrow. They occur first as nucleated cells, the nucleated red corpuscles, found only in the red marrow of the bones. These cells differ in structure with their age, and two extreme types may be distinguished,—one mature and ready to be converted to a non-nucleated corpuscle, and one immature, as shown by the char-

acter of the nucleus and the amount of hæmoglobin. This latter form multiplies by karyokinesis, and the daughter-cells sooner or later appear as mature nucleated red corpuscles, which then lose their nuclei by extrusion, and become non-nucleated red corpuscles. The biconcavity of the red corpuscles is probably caused in the first place by the removal of the nucleus from the middle of the spherical cell. The liberated nuclei go into solution in the blood plasma, and probably form or help to form the fibrinogen of the plasma. The immature or young nucleated red corpuscles are derived from spherical colorless cells, erythroblasts, having a definite histological structure and found in the marrow. These cells multiply actively by karyokinesis. The erythroblasts in turn are derived from larger embryonic cells, usually described in the adult as ordinary marrow cells. The structure of the nucleus differs from that of the erythroblast. The erythroblasts are not derived each from one of these larger cells by a process of condensation, but the embryonic cells multiply by karyokinesis, and the daughter-cells of the first or following generations acquire the structure of erythroblasts. The chief point in the paper is the proof that the mature nucleated red corpuscles lose their nuclei by extrusion, and not by absorption, in changing to the ordinary red corpuscle of the circulation. The act of extrusion can be observed in part in the living cells.

6. Very severe and sudden bleeding (in cats) is followed by the appearance in the circulation of red corpuscles containing a large fragment of nuclear material. This fragment persists until the corpuscle disappears. Apparently the greatly accelerated production of new corpuscles causes a too rapid extrusion of the nuclei, so that a portion remains entrapped in the corpuscle.

7. The apparent gemmation of non-nucleated red corpuscles from the nucleated forms, as observed by Malassez, is probably owing to the multiplication of the nucleated cell and the subsequent loss of a nucleus from one or more of the daughter-cells before the complete separation of the cells has been effected.

8. While the spleen of the adult mammal does not take part in the production of new red corpuscles under normal conditions, it may be made to resume this function in consequence

of prolonged or extreme anæmia produced by repeated bleedings.

9. The leucocytes of the blood are derived from the lymph leucocytes (lymphocytes). The latter enter the circulation as small corpuscles with vesicular nuclei and scanty protoplasm, and are not amœboid. They develop into larger cells, with finely granular protoplasm which possess amœboid movements. These have at first an oval vesicular nucleus, which afterwards becomes elongated and assumes a horseshoe or spiral shape. From this last form the multinucleated cells are derived by fragmentation of the nucleus. The fragmentation of the nucleus is probably followed by the disintegration of the whole cell.

10. The fragmented nuclei after the disintegration of the cell persist for a time in the circulation as the blood plates.

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EXPLANATION OF PLATE.

FIG. 1. Blood from the heart of a foetal cat, 2.7 cms. long, stained with methyl green, shows the large nucleated corpuscles (ancestral form) and the ordinary circular biconcave mammalian corpuscles. One of the latter is shown with its nucleus escaping.

FIG. 2. Shows the way in which the nucleus escapes from the nucleated red corpuscle. 1, 2, 3, 4, represent different stages of the extrusion noticed upon the living corpuscles; the drawings are colored to correspond with the rest of the figure. *a*. Specimen from the circulating blood of an adult cat bled four times. *b*. Specimens from the circulating blood of a kitten forty days old, bled twice. *c*. Specimens from the blood of a foetal cat 9 cms. long. Others from the marrow of adult cat, two of the figures showing the granules present in the corpuscle which have been interpreted erroneously as a sign of the disintegration of the nucleus. All the specimens stained with methyl green.

FIG. 3. Examples of apparent budding of the nucleated corpuscles, resulting from the extrusion of a nucleus from one of the cells after division. From the marrow of a cat. Stained with methyl green.

FIG. 4. Examples of the large nuclear granules found in the newly formed red blood corpuscles (cat) after severe and sudden bleeding.

FIG. 5. Multiplication of the nucleated red corpuscles. Methyl green. Marrow of young kitten after bleeding.

FIG. 6. White corpuscles and blood plates, stained with methyl green, from the blood of an adult cat, bled once to 90 cc., and treated with methyl green and acetic acid. To show the origin of the blood plates from the nuclei of the multinucleated leucocytes.

FIG. 7. Newly formed red corpuscles from section of marrow of femur in a foetal cat 9 cms. Shakespeare-Norris stain of indigo carmine. To show the granules with outline of nucleus seen in the newly formed corpuscles after extrusion of the nucleus and the dissolution of the hæmoglobin.

FIG. 8. Nucleated red corpuscles stained with methyl green, to show the mature and immature forms and the intermediate stages and the colorless erythroblasts.

FIG. 9. Nucleated red corpuscles from sections of the marrow, stained in hæmatoxylin, eosin, and saffronin, to show the preference of the nucleus of the mature form for saffranin, and of the immature form for hæmatoxylin.

FIG. 10. Karyokinetic figures of the nucleated red corpuscle, from a specimen of young marrow teased in Flemming's solution, and afterwards stained in saffranin.

FIG. 11. To show the origin of the erythroblasts and nucleated red corpuscles from the embryonic cells (marrow corpuscles). From the liver of a foetal cat 2.7 cms., teased in Flemming and stained in saffranin.

FIG. 12. To show the marrow corpuscles. *a* and *b* with oval nuclei, *c* and *d* with coiled nuclei, and *e*, *f*, with the protoplasm loaded with coarse granules. Specimens teased in Flemming and stained with saffranin.

FIG. 13. From a section of the liver of a foetal cat 2.7 cms., showing the development of the liver vessels and the nucleated red corpuscles. To the right of the figure the newly formed vessel contains a number of non-nucleated red corpuscles, surrounded in the section by the coagulated plasma.

FIG. 14. A second section from the same liver.

FIG. 15. White corpuscles from the blood of a young kitten bled once. Treated with methyl green and acetic acid, to show the origin of the multinucleated from the uninucleated forms.



OBSERVATIONS UPON THE OCCURRENCE, STRUCTURE, AND FUNCTION OF THE GIANT CELLS OF THE MARROW.

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THE observations which this communication is intended to record were made in the course of a study of the hæmatopoeitic function of the marrow, the results of which have been given in the previous paper. The giant cells have been supposed by some, notably by Foa and Salvioli (1), to have a direct connection with the production of new red corpuscles; so that an investigation of this last subject necessitated a more or less thorough consideration of the giant cells. My observation convinced me that no direct connection exists between the giant cells and the newly formed red corpuscles. The reasons upon which this conclusion is founded, together with certain interesting facts not heretofore observed relating to these peculiar cells, forms the excuse for the present brief paper, which for the rest does not attempt to give any final solution to the problem of their function in the marrow. My observations have been confined to the cat, as this was the animal which was found to be most convenient for the study of the blood corpuscles. The giant cells, moreover, seem to show certain differences in structure in different mammals (Werner), so that by restricting the work to one animal the different preparations could be more easily compared amongst themselves.

Before attempting to give a description of these cells as found in the cat's marrow, it is necessary to insist upon a division of them into two classes,—a distinction which has been emphasized before by certain writers, but which is forgotten or denied by others. We have, in the first place, giant cells containing a variable number of separate nuclei. These form what are generally described as giant cells or myeloplques, and have been found not only in the marrow, but also

in a number of pathological formations, tubercle, syphilis, etc. In the second class, the cell contains not many, but one huge nucleus, often bent or coiled upon itself, or imperfectly segmented or notched so as to form a complicated structure.

These have been described as giant cells with budding nuclei (Bizzozero [2]); but it seems to me that they are worthy of a more distinctive name. I shall speak of them hereafter as megakaryocytes, or large nucleated giant cells, while the first class might be named polykaryocytes, or multinucleated giant cells. Some of the German histologists, especially Arnold (3), have held that transitional forms can be found between the two classes, and look upon them therefore as two stages in the life-history of a single cell. The first stage is supposed to be the large nucleated form, and this by fragmentation passes into the multinucleated form. My observations upon the cat have led me to believe that we have here two entirely different cells, probably with different functions, and that what are described as transitional forms, which I have also seen, though very rarely, are such only in appearance, and can be accounted for more easily upon other grounds, possibly as cells undergoing degenerative changes.

In the cat the polykaryocytes are found in great numbers in the developing bone of the fœtus, and are usually seen in sections lying upon the spicules of bone while in process of formation. In extra-uterine life they can be found also, but only in the marrow of the spongy bone, in the neighborhood of the bony dissepiments. I have never met with them, in the cat, lying in the mass of red marrow which fills up the interstices of the spongy bone and forms solid plugs at the ends of the medullary cavity of the long bones. It is well known, also (4), that when pieces of sponge or other porous substances are introduced into serous cavities, the leucocytes swarm into the interstices of the foreign body, and in a short while multinucleated giant cells are found lying upon the partitions, just as in the bone they lie upon the bony spicules.

Very many hypotheses have been made as to the origin of these cells, the chief question being whether they are derived from the growth of a single small cell or from the fusion of a number of separate cells. It is not necessary for me to give here any detailed account of the views that are held upon this

question, as I have no special observations of my own to offer which might help to solve the problem. My own opinion has been that this form of giant cell is produced by the fusion or amalgamation of a number of smaller cells. I have been led to this view chiefly from the fact, already mentioned, that they seem to be found only when lying upon some solid substratum, such as the septa of sponge or the spicules of spongy bone. In the latter locality, when the spicules of forming bone are covered with an epithelium of osteoblastic cells, it seems plausible to think that these closely packed cells might become forced to form a polykaryocyte, and a number of apparently transitional steps in this process can be seen in sections of the femur in foetal cats about 9 cms. long. The rows of osteoblastic cells are found in the same localities with the polykaryocytes, and the nuclei of the cells have the same vesicular character in both kinds of cells. As far, then, as my observations upon this cell have gone, they have induced me to side with those who believe they are derived from the fusion of small cells. The function of these cells is unknown. The common view that they are concerned in the absorption of bone (osteoclasts) seems to me to rest upon very slight evidence. If we find them in developing bone lying upon the cartilage trabeculae which are being absorbed, we find them also on the partitions of sponge or pith, introduced into serous cavities where no absorption is taking place; and the conclusion in the first case that the absorption which is going on is due to the giant cells (osteoclasts) is illogical. Absorption of tissues is an occurrence common enough in the body, and it is difficult to understand why the absorption of bone or cartilage should require the activity of a special cell, when the absorption of other tissues does not. It would seem more probable that this form of cell has no specific function, and that its formation is, in fact, accidental or, in a certain sense, pathological: that the presence of a solid substratum leads to an abnormally rapid growth of lymphoid cells, leucocytes, osteoblasts, as the case may be, and the fusion of some of these to produce multinucleated giant cells. The same explanation might hold, as far as I can see, to the occurrence of this form of cell in pathological formations, except that in these cases the too rapid growth is brought about by other conditions.

The large nucleated giant cell, or megakaryocyte, unlike the multinucleated form, is found, and found abundantly, in the midst of the red marrow filling up the ends of the long bones and the spaces between the trabeculæ of the spongy bone. It does not lie upon the spicules of bone or cartilage, but away from them, surrounded by marrow cells, nucleated red corpuscles, blood corpuscles, and the other cells characteristic of marrow. It appears in the marrow with its first formation, as shown by cross and longitudinal sections of the femur in an embryo cat 9 cms. long, and is then surrounded by the elements of the marrow. Throughout the rest of the animal's life it can be discovered in sections or teased specimens of the red marrow. It is evidently a peculiar kind of cell, which has some definite function to perform. Not only is it found in the marrow, but throughout embryonic life it is met with in abundance in the liver and the spleen as long as these organs have any distinct connection with the production of red blood corpuscles; and it occurs more abundantly, the more active the blood-forming function of the organ. In histological structure it is the same in the embryonic liver and spleen as in the adult marrow. This fact has been stated by others (Foa and Salvioli [1], etc.), and is perfectly evident to any one who will take the trouble to look. It is certain, therefore, that the function of this cell is the same in the embryo spleen or liver as in the adult marrow. That it is not simply one stage in the life of the multinucleated giant cell seems to be demonstrated by the fact that typical multinucleated cells are never found in the mass of red marrow, in the cat at least, lying in the cavity of the long bones, nor in the embryonic liver or spleen, though the megakaryocytes are so numerous.

The structure of the megakaryocytes has been clearly described by a number of observers, especially by Arnold (3). They are giant cells, each with a huge nucleus. The body of the cell is finely granular, and shows no special peculiarities in structure. It is interesting to note that it does not possess the power of amœboid contraction which is so marked in many of the marrow cells, especially those with elongated nuclei. The nucleus is the characteristic part of the cell, but varies considerably in size, complexity, and minute structure (Fig. 11, *a*, *b*, *c*, *d*). Frequently it is crescent-shaped, or even makes a ring;

at other times it is coiled upon itself, or appears simply as a large, central mass, with projections from its surface; but in most cases it shows incomplete constrictions of or partitions from the peripheral membrane or layer of chromatin, which tend to separate it into small nuclei comparable to those of the typical marrow cells. The nucleus is granular, and in well-preserved specimens shows a distinct chromatin reticulum, with conspicuous nodal points. In addition, one or many nucleolar masses may be present, sometimes one apparently for each incomplete small nucleus into which the large mass is divided, sometimes only one for the entire nucleus, or in some cases none at all. What I have called nucleolar masses or nucleoli are distinguished from the large granules or nodal points of the chromatin reticulum by their staining. In the triple stain of hæmatoxylin, eosin, and saffranin, the chromatin reticulum takes the hæmatoxylin stain as in nuclei generally, while the nucleoli, like the nucleoli of the marrow cells, show a preference for the saffranin, staining bright red when the time of exposure to the different stains is properly adjusted. In a number of instances, in the sections of the normal marrow of adult cats, megakaryocytes were met with in which the nuclei showed no chromatin reticulum at all, but stained diffusely or almost so with the different dyes employed, taking the stain like the nuclei of the matured form of nucleated red corpuscle described in my paper upon the development of the red corpuscles. In such cells where the chromatin was diffusely scattered throughout the nucleus it frequently happened that the nucleus was fragmented (Fig. 1, *e, f*). It seems to me that this appearance of the nucleus here as in the nucleated red corpuscles is a sign of old age and death, and that these cells are in process of dissolution, hence the fragmentation of the nucleus. Arnold takes the directly opposite view, and considers this appearance as one of the initial changes leading to a fragmentation of the nucleus and division of the cell into smaller marrow cells.

According to Arnold, the following successive changes in the structure of the giant cell occur, leading up to its fragmentation. 1. The first stage is characterized by an increase in the chromatin substance, the chromatin filaments become more numerous, form networks, etc., and toward the end of the stage

a division of the chromatin occurs, leading to a more or less diffused coloration of the nucleus. 2. The periphery of the nucleus becomes indented, and in many cases there is such an important increase in the diffused chromatin substance that the filaments can no longer be distinguished. The indentations of the periphery of the nucleus occur at many points and advance toward the middle, forming the complicated nuclear figures so characteristic of these cells. 3. The chromatin substance becomes concentrated at different points, forming small, dark-colored nuclear bodies which are united by colorless bands. By a continuation of these changes a number of entirely independent nuclei are formed. 4. The protoplasm segments round the newly formed nuclei, either endogenously or by constriction from the periphery. This makes up the process designated by Arnold as "indirect fragmentation," and it is, according to him, the normal method of development or multiplication of the giant cells. He admits in addition, and quotes from Martin and Waldstein to support the statement, that the multinuclear giant cells may reproduce also by true mitotic division of the nucleus, but thinks that this method of division is very rare. I have never myself seen any indication whatever that the nucleus of the giant cells divides by karyokinesis, though I have examined many sections of marrow from cats of all ages, normal, bled, and starved, so that with this animal at least it must be an exceedingly rare occurrence. Moreover, my observations upon the giant cells have never given me any evidence of the correctness of Arnold's view that these cells normally undergo indirect fragmentation. Foa and Salvioli also thought that the megakaryocytes break up by segmentation to form a number of colorless or hyaline cells, which in turn develop into nucleated red corpuscles, and I shall speak further of their theory in discussing the function of this form of giant cell.

On the other hand, that the megakaryocytes multiply by division, like other cells, giving rise to two daughter giant cells, has been clearly proved by my sections. In quite a number of cases the sections have shown me megakaryocytes with two large nuclei at the ends of the cells, and a constriction beginning between them, or, more frequently, two megakaryocytes lying side by side with the line of demarcation between them

complete, but the cells still adherent to each other (Figs. 2 and 3). Similar appearances have been seen and figured by Werner (5). In none of the cases of division observed by me was there any indication of karyokinetic figures; hence it is fair to conclude that this form of cell multiplies by direct division. The clear proof furnished by these observations that it does increase by division is also another indication that the megakaryocyte is a definite cell form, and not one stage in the formation of the multinucleated giant cell.

What now is the origin and function of this cell? Believing that the megakaryocyte has no genetic connection with the polykaryocyte, the theories of the origin of the latter have no bearing upon the former. My sections, especially those made through the femur of a foetal cat, 9 cms., at a time when the marrow was just beginning to form, gave me a number of apparently transitional forms between typical megakaryocytes and the small marrow or embryonic cells. A series of drawings, showing apparently the gradual development of the small cell into the giant cell, is given in the Fig. 4. The drawings were made from different portions of the section, and the theory they suggest is that the small cell enlarges, the increase affecting both the nucleus and the cell substance, and, after reaching a certain size, indentations of the periphery of the nucleus appear, or in many cases ingrowths of the peripheral chromatin, which give the incompletely segmented appearance to the nucleus that is so characteristic, and which has given to them the name of giant cells with budding nuclei. The megakaryocyte is not formed, then, by the fusion of a number of smaller lymphoid cells, nor from a single cell which increases in size by engulfing other similar cells. Both these theories might apply to the polykaryocytes, but certainly not to the megakaryocytes. These latter are developed by the steady growth in size of a smaller lymphoid cell, and the curious structure of the nucleus follows after it has reached a certain size in consequence of partial constrictions or divisions, which are never carried so far, however, as to lead to a complete separation.

With reference to the function of these cells, several different views have been proposed. Arnold and others, who believe that the megakaryocyte becomes ultimately a multinucleated

giant cell, believe that the latter constricts off or separates into smaller marrow cells. From this standpoint, the function of the large nucleated giant cell is simply that it forms one stage in a peculiar method of development of the lymphoid cells of the marrow. Löwit (6) speaks of the giant cell—including both varieties—as having some connection with the degenerative changes of the leucocytes, though as far as I know the exact nature of the relationship is not described. He gives three reasons for this view. 1. They are less frequent in the embryo than in the marrow of the adult, and the younger the embryo, the fewer the number found in the liver and spleen. The first part of the statement I cannot corroborate, as in the embryo liver, especially when at the maximum of its hæmatopoietic activity, the giant cells (megakaryocytes) are quite as numerous as in the adult marrow. The second portion of the statement is true to a certain extent. The number of giant cells (megakaryocytes) varies directly with the blood-forming activity of the organ, so that they are not numerous in the liver at its first formation nor toward the end of foetal life. 2. He has never been able to find them in the lymph glands, even after severe bleeding. In this, Löwit is confirmed by a number of other observers who have stated their inability to find giant cells in the lymph glands. Indeed, this assertion may be accepted as satisfactorily demonstrated, but its bearing upon Löwit's theory of a connection of the giant cells with the degeneration of the leucocytes seems to be very remote. While it is true that we do not find in the lymph glands either giant cells or degenerating leucocytes, that does not in any way prove that the giant cells have any relation to the degenerative changes of the leucocytes. 3. In sections of the marrow, the giant cells are not found where erythroblasts and leucoblasts are in active formation, but rather where the latter show signs of degenerative changes. This statement I cannot confirm; indeed, it seems to me that the reverse is true, as far, at least, as the erythroblasts are concerned, while with the leucoblasts I have not been able to notice that in the neighborhood of the giant cells there is any increase in the number of them undergoing degeneration. Foa and Salvioli have thought that they were able to demonstrate a connection between the giant cells and the nucleated red corpuscles. Their view, as

has been stated, is that the giant cell breaks up into a number of hyaline cells, — erythroblasts, to use Löwit's terminology, — which in turn develop into nucleated red corpuscles. Their strongest evidence for this view is the fact that whenever, in the foetus or in the animal after birth, there is an undoubted formation of nucleated red corpuscles, there the giant cells — megakaryocytes — are also found. This connection has been noticed by a number of observers, and is certainly very constant and striking. In the embryo liver, the embryo spleen, the adult marrow, and in the spleen of the adult during regeneration after partial excision (7) we find megakaryocytes and nucleated red corpuscles side by side. Nevertheless, I have never been able by the most careful and thorough observation to find any actual connection between these two histological elements, or between the giant cell and the erythroblast. Foa and Salvioli picture a group of nucleated red corpuscles supposed to be derived from the breaking up of a megakaryocyte, but groups of the kind figured are in reality derived from the multiplication of nucleated red corpuscles by division. Arnold has stated that white corpuscles are constricted off from the giant cells, but supposes that these corpuscles are not progenitors of the nucleated red corpuscles. In my own sections and teased preparations I have never been able to find any indication that the nucleated red corpuscles are budded off from the megakaryocytes, and no satisfactory example of the derivation of a lymphoid corpuscle of any description from them. In the marrow of the adult, after repeated hemorrhages, where the production of red corpuscles has been vastly accelerated, one would surely expect to see some undoubted sign of the derivation of the nucleated red corpuscle or its colorless predecessor from the giant cell, if it is the function of this last cell to serve as the origin of the new red corpuscles that are being formed. My failure to find any perfectly clear examples of such a derivation has compelled me to believe that the megakaryocytes take no direct part in the production of new red corpuscles.

In a few cases I have obtained giant cells evidently belonging to the class of megakaryocytes in which a smaller portion of the nucleus seemed to be completely separated from the main mass and was lying free in the cell, but always in such cases there was some possibility that the appearance was de-

ceptive. I was not able to obtain a sufficient number of clear cases to convince me that this is a normal occurrence in the life of these cells, though at the time I was convinced that their most probable function was to form the erythroblasts or progenitors of the nucleated red corpuscles; indeed, I abandoned the theory reluctantly because the evidence seemed to be opposed to it, or at least did not support it. If we adopt the compromise view that the giant cells furnish some of the erythroblasts while others, and probably most of the others, arise in a different way, then we could understand why in rapid regeneration of the blood after bleeding it is so rare to find giant cells in the act of producing erythroblasts. But it does not seem probable, to me at least, that these cells should be produced in one organ by two different methods. Nevertheless, the constant presence of megakaryocytes in the blood-forming organs induces me to believe that they have some function to perform in connection with the formation of blood. This is rendered more probable by the fact that in the embryo, at least, the megakaryocytes can be found in the newly forming blood-vessels surrounded by developing blood corpuscles. I have found this in sections of the liver of an embryo cat where, as has been described in another paper, the nucleated red corpuscles and the erythroblasts lie in cords which are destined to become the future blood-vessels of the liver. In some cases, in fact, the cords may be seen to end in channels filled with coagulated plasma and red corpuscles, with or without nuclei. Now, in these cords of blood cells I have found the megakaryocytes, showing that they are connected in some way with the blood (Fig. 5). In longitudinal sections of the hind leg of the same embryo I have found developing blood-vessels lying among the embryonic muscle fibres and in the blood-vessel giant cells, — megakaryocyte, — as shown in Fig. 6. It is very hard to understand what this cell is doing in such a place if it is not connected with the production of either the formed elements or of some of the chemical constituents of the blood.

As I have just said, I cannot find any corroborative evidence for the first view, and am therefore inclined to look favorably upon the second; namely, that the function of the megakaryocyte is to manipulate, in some way, the material of the plasma or lymph, forming some substance for the nourishment of the

developing blood cells. This view was suggested to me by a curious phenomenon which I have occasionally found in connection with these giant cells, and which, so far as I know, has not been noticed before. In many sections of the marrow, but especially in sections through the bone and marrow of the femur of a foetal cat (9 cm. long), I have seen the megakaryocytes, either singly or in groups, with a delicate reticulum radiating out from them on all sides, and enclosing within its meshes the other elements of the marrow. A sketch of this appearance is given in Fig. 7. I should add that Werner has described but not figured what appears to be the same reticulum. In the young developing marrow this appearance is so common and so striking that I thought at first the megakaryocytes had for their function the formation of a supporting reticulum for the marrow, secreting it, as it were, from the cell substance. But when examinations were made of teased specimens of the fresh marrow of young kittens to find if possible whether a giant cell with its reticulum could be teased out from the other elements, I obtained the cells, surrounded not by a reticulum, but by a very large envelope of exceedingly fine and pale material (Fig. 8). Round the nucleus of the cell was the ordinary granular protoplasm forming the body of the cell, but outside of and surrounding this was a large envelope of much more delicate and hyaline material, which did not stain with methyl green. As this was watched under the microscope, in a very dilute NaCl solution of methyl green, vacuoles began to form in it (Fig. 9), and becoming rapidly larger, finally made a reticulum such as I had found in my sections surrounding the cell. This convinced me that the reticulum seen in the sections arose from the action of the fixing and hardening reagents upon this secretion from the cell. The theory that the giant cells make a reticulum is rendered improbable, also, from the fact that they occur in the developing blood-vessels of the embryo. In many cases in the teased specimens the action of the reagent had gone so far that the giant cells were found surrounded only by vesicular-like bodies arising from the vacuolation of the secreted material, as shown in Fig. 10. It seems to me that this broad envelope of material surrounding the megakaryocyte, and evidently formed by it, is very significant. As I found it, no nuclei were scattered

through it, and hence the most natural explanation is that it is a material secreted by the cell which is finally dissolved in the plasma, and is used, possibly, for the nutriment of the blood-forming cells; though this, of course, is mere speculation. In sections nothing remains of the material except the reticulum, and this does not stain with any of the reagents used, — alum carmine, hæmatoxylin, eosin, saffranin, Ehrlich-Biondi's stain, indigo carmine (Shakspeare-Norris stain), — or at least stains much more feebly than the protoplasmic cell substance.

SUMMARY.

The contents of the paper may be summed up briefly as follows:

1. Giant cells fall into two classes: *a*. Polykaryocytes, or multinucleated giant cells found in developing bone, in pathological formations, or porous bodies kept in lymph cavities, etc.; *b*. Megakaryocytes, or large nucleated giant cells found in the red marrow of the adult and in the blood-forming organs, liver, spleen, etc., of the embryo.

2. The polykaryocytes have no special function, are not related to the megakaryocytes, and are formed by the fusion of smaller cells in consequence of too rapid growth.

3. The megakaryocytes form a peculiar class of cells. They arise from the growth of small lymphoid cells, and afterwards reproduce by direct division. During their life they form a secreted material which can be seen for a time by the microscope, but finally dissolves in the plasma.

They seem to take no direct part in the production of nucleated red corpuscles or erythroblasts. After a certain period the nucleus alters in such a way that it stains diffusely and then fragments. This seems to be a degenerative change, and probably ends in the total disintegration and dissolution of the cell.

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EXPLANATION OF PLATE.

FIG. 1. *a, b, c, d.* Drawings of megakaryocytes, to show some of the variations in structure of the nucleus. *e, f.* Megakaryocytes in which the nucleus stains diffusely and fragments into smaller pieces, explained as degenerative changes.

FIG. 2. Three megakaryocytes; two in the act of dividing; from a camera lucida sketch.

FIG. 3. Two megakaryocytes; division complete, but the cells still connected; from a camera lucida sketch.

FIG. 4. Four cells from the developing marrow in a section of the femur of a foetal cat 9 cms. long, stained with saffranin, and intended to illustrate the development of a megakaryocyte from a marrow corpuscle.

FIG. 5. Section of the liver of a cat embryo 2.7 cms. long, showing a megakaryocyte lying in a developing blood vessel, and surrounded by erythroblasts.

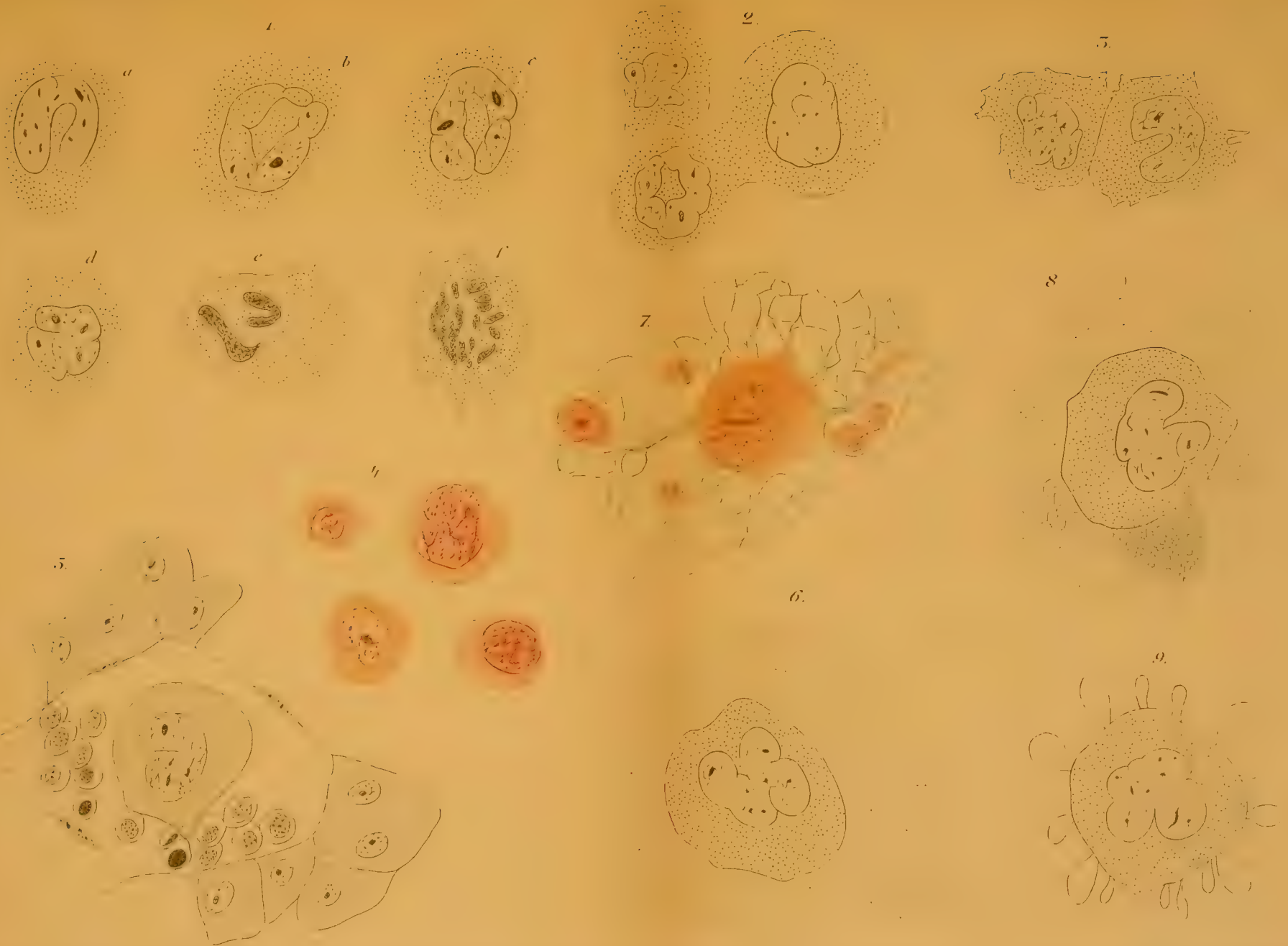
FIG. 6. Section of the hind leg of the same embryo, to show a megakaryocyte lying in a developing blood-vessel of the muscular tissue. On one side there is still a solid cord of erythroblasts, with some nucleated red corpuscles; on the other the blood plasma and fully formed nucleated red corpuscles lie in contact with the giant cell.

FIG. 7. Camera lucida sketch from the section of the femur of the foetal cat 9 cms. to show the reticulum radiating from the megakaryocytes found in the marrow.

FIG. 8. A megakaryocyte surrounded by its broad envelope of secreted material from a preparation of the marrow of a young kitten, teased in a weak solution of methyl green in normal salt.

FIG. 9. The same cell, showing the vacuolation that takes place in the enveloping substance. The vacuoles at first small, as on the under side of the cell, become larger, until they form a structure resembling somewhat the reticulum shown in Fig. 7. The sketches of the vacuolation were made at different times, and have been shown in the same figure to indicate the gradual growth.

FIG. 10. A megakaryocyte from a similar preparation, in which the action of the reagent had gone so far before the cell was examined, that a number of vesicles adhering to the cell was all that remained of the original envelope.



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CONTRIBUTIONS ON THE MORPHOLOGY OF THE ACTINOZOA.

I. THE STRUCTURE OF *CERIANTHUS AMERICANUS*.

J. PLAYFAIR McMURRICH.

THE genus *Cerianthus* was established in 1829 by Delle Chiaje for the Mediterranean form which we now know as *C. membranaceus*, it having been originally described by Spallanzani as *Tubularia membranacea*. Until 1854, however, no thorough study of the internal structure was made, but in that year appeared the excellent memoir of Haime ('54). In this it is shown that each "loge" has communicating with it two tentacles, one belonging to the marginal, the other to the oral group. Haime also described the arrangement of the mesenteries, showing that two mesenteries, the cavity between them forming a continuation of the "fossette gastrique" (siphonoglyphe), extend the entire length of the body to the terminal pore, while the rest stop at a short distance below the internal opening of the stomatodæum, and are unpaired, although they are alternately slightly unequal in length and prominence. Haime described, too, the hermaphroditism of this species, and gave an incomplete account of some stages in its development. His account of the histology was, however, by no means exhaustive, though admirable, when the facilities for such work at that time are taken into consideration.

For twenty-five years nothing further was done towards the

elucidation of any members of the genus *Cerianthus*, but in 1879 two papers of importance appeared. The brothers Hertwig ('79) in their studies on the nervous system of the Actiniaria, examined histologically *C. membranaceus* and *C. solitarius*, and added much to our knowledge of the minute anatomy of these forms, discovering the nervous tissue, describing the arrangement of the muscle-cells correctly, and showing the similarity of all the tissues to those of the other groups of Actiniaria. As regards the general structure, however, they made no advance upon what had been done by Haime, not even correcting some of the errors into which that author had fallen.

The other paper of 1879 was by von Heider, who treated *C. membranaceus* in as thorough a manner as he had previously done *Sagartia troglodytes*. Where the Hertwigs are lacking, von Heider excels, giving a more correct account of the anatomical features of the species than Haime had done, but, his treatment of the histology is in some points not so complete. As regards the anatomy, he showed that the pair of elongated mesenteries are not the most ventral, but that between them is a pair reaching the wall of the siphonoglyphe, but terminating a very short distance below the margin of the groove. These are the ventral directives. He also extended Haime's discovery as to the alternating inequality in length of the mesenteries, by showing that as a rule there is an alteration of gonophoric and non-gonophoric mesenteries, and accordingly divided the mesenteries into three groups; namely, (1) Filament septa, which are non-gonophoric; (2) Genital septa; and (3) Continuous septa, which are represented only by the single pair which reaches the terminal pore. Von Heider describes the Filament septa as giving rise to the acontia, while the Genital septa are provided with mesenterial filaments (craspeda, Gosse). The Hertwigs, in a supplement to their description, after confirming several of von Heider's results, criticise this differentiation of the filaments in the two groups of mesenteries, stating that "in der Beschaffenheit der Mesenterialfilamente zwischen beiden kein Unterschied vorhanden ist." It will be seen that, so far as the structure of the filaments is concerned, this is true also for *C. Americanus*, though there is a slight difference in the arrangement of the different parts of the filament.

In 1880 a paper by Jourdan ('80) appeared, written, however,

before the publication of the contributions of the Hertwigs and of von Heider. It adds nothing to Haime's description of the general structure, and falls much behind the Hertwigs' contribution in the treatment of the histology.

In 1888 a paper by C. Vogt ('88) was published, in which was confirmed the supposition of the Hertwigs that new mesenteries are formed in *Cerianthus* solely at the dorsal surface, in the region which corresponds in other orders of Actiniaria to the intra-mesenterial space bounded by the dorsal directives. Vogt also calls attention to the unpaired tentacle corresponding to the ventral intra-mesenterial space, which had previously been observed by Haime in *Cerianthus* and by A. Agassiz ('62) in *Arachnactis*, and demonstrated the close relationship existing between these two genera.

Later in the same year, H. V. Wilson ('88) added to Vogt's observations by showing a similar method of formation of new mesenteries in a free-swimming larva of an unidentified species of *Cerianthus* obtained at Nassau, Bahama Islands, W.I.

In the following year Fischer ('89)¹ again called attention to the unpaired ventral tentacle, and to the bilateral symmetry of *C. membranaceus*.

Up to this time no accounts of the internal structure of any other species of *Cerianthus* had been given. The Hertwigs state that they studied *C. solitarius*, but make no special statements concerning its general structure. In 1889, however, Danielssen ('89) published an account of the structure of a Norwegian *Cerianthus*, which he termed *C. borealis*,² and which presents many important variations from the structure of *C. membranaceus*. The principal anatomical peculiarities are the small number of mesenteries, sixteen only, the occurrence of either ova or spermatozoa only in any individual, and the difference in the arrangement of the mesenteries in the males and females, all the mesenteries in the latter extending to the terminal pore, except the ventral directives, which stop a short distance above it, while in the males the arrangement is much more similar to what is found in *C. membranaceus*.

¹ The original paper I have not seen; my knowledge of its contents is derived from the abstract in the Journal of the Royal Microscopical Society, December, 1889.

² It seems probable that this form, which Danielssen holds to be distinct from *C. Lloydii*, is not the same as Verrill's *C. borealis*, described in 1873. In this case Verrill's application of the name has the priority, as Danielssen's description was not published until 1877.

CERIANTHUS AMERICANUS, L. AGASSIZ.

The earliest mention of this form is by L. Agassiz ('59), who states that it was found by H. James Clark, in 1852, at Charleston, S.C., where it lives in tubes sunk in the mud-flats of the harbor. It is referred to the genus *Cerianthus*, but no specific name is given. Agassiz observed the terminal pore, which he terms the anus, and states that the upper parts of the mesenteries bear female reproductive organs, and the lower parts male organs.

The specific name is stated by Verrill ('64) to have been bestowed in manuscript by Agassiz, and Verrill gives the first full description of the species, from drawings made for Agassiz by his artist, Burkhardt, and from alcoholic specimens in the Museum of Comparative Zoölogy at Harvard College.

In a subsequent paper Verrill ('72) records its occurrence on the coast of North Carolina, where it was collected by Dr. Yarrow, and where it had previously been found by Stimpson, but adds nothing to the description given in 1864.

Among the "Challenger" material R. Hertwig ('82) found a single specimen of a *Cerianthus* obtained in thirteen fathoms in the mouth of the Rio de la Plata. He identifies it with *C. Americanus*, but unwilling to mutilate the single specimen, did not investigate it anatomically.

Finally I added ('87) a few points to the general description, but gave no account of the internal anatomy and histology which have never hitherto been examined.

I. EXTERNAL FEATURES.

The specimens of *C. Americanus* which I studied were found at Beaufort, N.C., where the Summer Station of the Johns Hopkins University was located in 1885. In the shallow sounds there the bottom is largely very dark mud, sometimes with a superficial coating of sand. Large areas of such mud are uncovered at low tide, forming what are termed the mud-flats, and it is on these flats that *Cerianthus* is found, usually just below the average low-tide mark. It lives in cylindrical burrows extending, usually at an angle, downwards for some distance, how far I was not able to determine. Like other members of

the genus it secretes a case, open at both ends, and composed of hardened mucus and nematocysts. Animals removed from the case and kept in an aquarium rapidly secrete for themselves a new habitation, which, however, is naturally thinner than the original one and of a lighter color, being almost white, while normally the tube is dark gray, due to staining by the black mud with which it is in contact. The inner surface is purplish gray, being tinged by the pigment of the animal.

The largest specimen I obtained measured about 20 cm. in length, with a diameter at the middle of 1.5–2 cm., and at the disc of 1.8–2.5 cm. The outer tentacles measured 3.4 cm., while the oral series measured 1–1.2 cm. These measurements fall very short of those given by Verrill, who states that the largest specimens in expansion measure 60–70 cm. in length, with a diameter at the disc of nearly 4 cm. and at the middle of the column of 2.5 cm. My preserved specimens measure 5.5–6 cm. in length, and about 1.5 cm. in diameter.

The color of the column is some shade of brown (Pl. VI., Figs. 1 and 2), varying from pale chocolate-brown to deep purplish brown. The upper part is always darker than the lower, and in some cases the column is marked with longitudinal lines of a lighter shade than the ground color. The marginal tentacles are of a paler brown than the column, except the outermost, which are purplish blue. The oral tentacles in all the specimens I observed were pure white; Verrill, on the other hand, describes them as being darker than the marginal ones, and marked with white longitudinal lines. In the Beaufort specimens, however, the tentacles of both series are unmarked by lines, spots, or annulations. The disc is yellow with white lines crossing it radially.

The column is cylindrical and smooth, tapering gradually towards the posterior extremity, which is rounded, and bears a small terminal pore. The marginal tentacles vary somewhat in number. Verrill states that they are 125 or more, but in the Beaufort specimens they did not amount to 100, varying, according to the counts made, from 89 to 94 (95?). We know from the observations of Vogt and Fischer that there is always an odd number, the unpaired tentacle corresponding to the space bounded by the ventral directives. The absolute number of the tentacles perhaps increases throughout the entire life of the animal, and

the discrepancy between the number found in the Beaufort specimens and that given by Verrill is probably in accord with the smaller size of my specimens. A tentacle of the oral series corresponds to and is opposite each marginal tentacle, and both series seem to be arranged in three cycles, not four, as I stated in my previous paper, but I was not able to ascertain the relations of the various cycles in the two series. The accounts given by von Heider ('79) and Fischer ('89) of these relations in *C. membranaceus* differ materially. The number of cycles, however, does not have the same significance here that it has in the Hexactiniæ, and the arrangement of the tentacles in cycles is no doubt, as von Heider suggests, altogether mechanical, and due to crowding, and accordingly their relation in the marginal and oral series, and the number of the cycles in which they are arranged, may vary.

2. INTERNAL STRUCTURE.

On laying open a specimen of *C. Americanus* by a longitudinal incision, the appearance presented is that indicated in Figure 1, Pl. VII., in which, however, the disc and tentacles have been omitted. In the upper part one sees the stomatodæum with a well-marked longitudinal groove — the siphonoglyphe. By close examination it may be seen to be finely grooved longitudinally, each groove corresponding to a mesentery; a few transverse grooves may usually be seen, but they are more or less irregular, and are no doubt due to contraction. Its lower margin is usually reflected, so that a transverse section in this region gives the appearance represented in Figure 3.

The mesenteries are arranged on a very different plan for what has been described for *C. membranaceus* and *C. borealis*, Danl. On first laying open a specimen of *C. Americanus*, one sees that more than one pair of mesenteries reach the posterior extremity of the body, and yet all do not extend so far. In the specimen figured twenty-three well-developed mesenteries are present. The total number of mesenteries is really ninety-two, as will be seen later, but of these only twenty-three pass more than half-way down the column, and it is to the arrangement of these that I wish first to direct the attention. Extending the entire length of the body from the region of the siphonoglyphe is a pair of mesenteries corre-

sponding to the similar pair in *C. membranaceus*, which von Heider terms the Continuous septa. Between these, and in reality constituting the ventral directives, is a pair, as in *C. membranaceus*, which are very short, and hardly extend below the level of the lower opening of the stomatodæum. Next to the ventral continuous mesenteries on either side comes a mesentery which only extends a short distance beyond the middle of the column. Dorsal to it come three continuous mesenteries, the middle one of which, however, hardly reaches the extremity of the body. Then follow two extending slightly beyond the middle of the column, and similar in length therefore to the one immediately succeeding the ventral continuous mesenteries: and succeeding these is a single continuous mesentery. So far the symmetry has been perfect, and I have found the arrangement here described to hold in another specimen which I studied. Unfortunately, the third specimen I had for investigation was not favorable for the examination of the mesenteries.

The succeeding mesenteries passing dorsally on either side vary from the regular arrangement. They are more recent in date of formation than those towards the ventral line, and may not yet have reached their final development, or may remain in this somewhat immature condition. On one side, dorsal to the continuous mesentery last mentioned, there is another similar to it, but on the other side occurs one belonging to what may be termed the second grade, reaching only to about the middle of the column. Occupying the dorsal region are four mesenteries, all of the second grade, two alternate ones, however, being slightly longer than the other two. Upon what is the left side of the figure a mesentery, also of the second grade, was detached in making the longitudinal incision, and was omitted from the drawing. The last mesentery of the second grade on the left side is the youngest of those of the first two grades, being nearest the median dorsal line.

I have denoted the mesenteries so far described according to their length as the first and second grades, the latter being the shorter ones which extend only about half-way down the column. In the figure (Fig. 1) these mesenteries are the only ones represented for the most part, but three (3) still smaller than those of the second grade are indicated. In reality, the mesenteries of this third grade alternate with those of the first and

second grade, and there are consequently twenty-three of them. These third-grade mesenteries extend only a short distance below the internal opening of the stomatodæum, not more than a centimetre, and usually less than that. Like the longer ones, they are perfect in their upper part.

Figure 2, Pl. VII., is a semi-diagrammatic representation of the mesenteries of the various grades, and it will be seen from this that there is still a fourth grade of mesenteries, shorter than any that have hitherto been described, and alternating with the mesenteries of the other three grades. There are, therefore, forty-six of them, and altogether in all the grades, accordingly, there are ninety-two mesenteries, one more than the number of tentacles, marginal or oral, of which I counted ninety-one. These fourth-grade mesenteries hardly reach below the lower opening of the stomatodæum, and are not readily seen in a preserved specimen, being usually overlapped by the adjoining mesenteries, and further concealed by the tangled mass of acontia which arise from the edges of the mesenteries just below the stomatodæum. The ventral directive mesenteries, as already mentioned, belong to the fourth grade.

Figures 3, 4, and 5 show some interesting features in the relations of the mesenteries of the four grades. They are transverse sections of the ventral region of the column in its upper part. Figure 3 passes through the stomatodæum shortly above its lower extremity, cutting its reflected portion. The ventral siphonoglyphe (*si*) is readily made out, the ectoderm lining it not being thrown into folds as it is elsewhere. The ventral directives (*D*) are still in connection with the stomatodæum, but the other mesenteries have separated from it. Sections a little higher up show that all the mesenteries are perfect; but the ventral directives retain their connection with the stomatodæum throughout a greater portion of its length than do the others. The mesentery (1) immediately adjoining the directives is one of the ventral continuous mesenteries, which at this level are narrow, as are all the mesenteries immediately below the point where they lose connection with the stomatodæum. Succeeding it comes a mesentery of the fourth grade (4), and following this one of the third grade (3), both of the same width as the ventral continuous mesentery. The mesenterial filaments of these three have the same structure, being

somewhat bilobed, constricted by a well-marked "neck," and evidently comparable with the "Flimmerstreifen" of the mesenterial filaments of the Hexactiniæ. The section has not cut all the mesenteries at the same relative level, so that those furthest from the ventral median line show features which are to be found in 1, 4, and 3, a little farther down. The mesentery (4') which succeeds 3 is again of the fourth grade. It is much wider than those nearer the middle line, and its mesenterial filament is quite different, appearing simply as a small rounded knob at the free edge of the mesentery not separated distinctly from the endoderm by a neck, and corresponding to the "Nesseldrüsenstreif" of the Hexactiniæ. A few sections higher up this mesentery, and the other mesenteries of the fourth grade represented (4'' and 4'''), which resemble it in width and structure, are exactly similar in all points to 4. The three mesenteries which alternate with 4', 4'', and 4''' are of the second (2), third (3'), and first (1') grades, and exactly resemble 1.

Figure 4 is a section nearly 2 mm. below that just described. The ventral directives, only one of which is figured (*D*), have practically disappeared, being indicated simply by a slight elevation of the mesogloea. The mesenteries of the first, third, and second grades nearest the middle line (1, 3, and 2) are broader than they were higher up, but still retain the same kind of filaments they possessed there. The more external (dorsal) mesenteries are much wider, and, in fact, have now reached their final width, and ova have begun to appear in their mesogloea. Their mesenterial filaments have not been represented, but they are still of the same nature as they were higher up. The mesenteries of the fourth grade (4, 4', 4'', and 4''') have lost all trace of their mesenterial filaments, and have become very narrow.

Figure 5 is from a region about 1.5 mm. below the preceding figure. The mesenteries of the fourth grade have now disappeared, being represented, like the ventral directives, only by slight projections of the mesogloea. The mesenteries of the first three grades still persist; all are gonophoric, but all have lost their mesenterial filaments.

Still further down, in a section taken about 1 cm. lower, the mesenteries of the third grade (3 and 3') would have dis-

appeared, and in a section slightly below the middle of the column only the mesenteries of the first grade would be found.

In a section a little higher up than the third (Fig. 5) of those figured, on the gonophoric mesenteries, just before the mesenterial filaments die out, a very small portion of the "Nesseldrüsenstreif" can be seen. It is histologically like the same portion in the mesenteries of the fourth grade, but does not reach anything like the development it has upon these latter mesenteries, being of very small extent, and somewhat apt to be overlooked.

It will be seen from what has been said that the mesenteries of the fourth grade are noticeably different from those of the other three grades with which they alternate. They are much shorter; they never bear reproductive elements; and they possess both the "Flimmerstreifen" and the "Nesseldrüsenstreif" of the mesenterial filaments well developed. On the other hand, the mesenteries of the first three grades are all gonophoric, and their filaments consist almost entirely of the "Flimmerstreifen," the "Nesseldrüsenstreif" being very short.

C. membranaceus has been found to be hermaphrodite by all who make statements on this point, the ova and spermatozoa being both present upon all the gonophoric mesenteries. *C. borealis*, Danl. is, according to its describer, bisexual, and *C. Americanus* agrees with it. Agassiz, as already noted, states that this last form is hermaphrodite, the ova occurring in the upper part of the mesenteries, and the spermatozoa lower down; but this is certainly not the case in the three specimens I had for study. All were females, ova only occurring in the gonophoric mesenteries, and sections taken at varying distances down the column to within 2 mm. of the posterior extremity show no trace of spermatozoa.

Of course it is possible that *C. Americanus* may be dichogamous, as Lacaze-Duthiers ('72) believes the Hexactiniæ to be. In all the Actinians which I have examined, amounting to over fifty species, with the exception of certain Zoantheæ, which are known to be hermaphrodite, I have never found any trace of dichogamy or hermaphroditism. If dichogamy occurred as a rule, one would expect to find occasionally, at any rate, some traces of spermatozoa associated with ova, or of ova with sper-

matozoa; but this, so far as I know, never occurs in any of the Hexactinians. I believe, therefore, that *C. Americanus* is really bisexual, and not dichogamous.

3. HISTOLOGY.

The maceration of fresh tissues gives the most satisfactory results as to the structure of the histological elements in the Actiniaria. This method I was unable to employ, and the maceration of preserved specimens gave as usual unsatisfactory results. Nearly all the facts I have to present have been derived from the study of sections, and are therefore somewhat fragmentary. They suffice, however, to show a very close similarity in the histology of *C. Americanus* to that of *C. membranaceus*, as described by the Hertwigs ('79), von Heider ('79), and Jourdan ('80), and, on the other hand, considerable differences from what Danielssen ('89) has described for *C. borealis*, Danl.

(a) *Tentacles and Disc.*

The ectoderm in these parts is covered by a very distinct cuticle, which shows a dotted appearance, produced probably by the existence of perforations for the passage of the cilia. The outer portion contains numerous nematocysts which stain deeply with borax-carmines and are cylindrical or slightly curved with the filament spirally coiled. They resemble those described by von Heider and Haime in the same situations. Two kinds of gland cells are present; one resembles goblet cells, and are by far the most abundant, the other kind occurring only sparingly, and being of the structure figured by the Hertwigs (Pl. VIII., Fig. 15, *d*) and by Jourdan (Pl. XII., Fig. 85, *g*). I could not observe that the latter kind were more numerous in the oral tentacles than in the marginal as Jourdan describes, the histological structure of both series of tentacles being identical. In the disc, however, they do seem to be more abundant than in the tentacles. In sections which were slightly torn I could perceive indications of the presence of "Stützzellen" and sensory cells, but maceration preparations are necessary for their proper study.

Below the epithelial layer to which these structures belong comes the nerve layer. In the tentacles the nerve fibrils are

few and not readily distinguishable; but in the disc they are much more distinct, and form a well-marked band in sections (Pl. VII., Fig. 6, *n*). This difference in the development of the nerve tract is in correlation with the development of the longitudinal muscles in the two regions. Occasional nuclei can be distinguished in the nerve region, which are probably the nuclei of ganglion cells; they are no larger, however, than the nuclei of the cells of the epithelial layer. They appear to be more numerous in the disc than in the tentacles.

The longitudinal muscles have the same development as in *C. membranaceus*. In the tentacles they cover slight elevations of the mesogloea, and are arranged in a single layer; in the more contracted tentacles they appear to form two layers, the fibrils of the upper layer alternating with those of the lower, but they never show so extensive a development as that figured by Jourdan (Pl. XII., Fig. 83). On the disc, however (Fig. 6, *lm.*), they are arranged on both sides of delicate lamellæ of the mesogloea, which are arranged "like the leaves of a book," the entire muscle having a thickness of a 0.032 mm. I could find in the oral tentacles no trace of the ectodermal circular muscles described by Jourdan from maceration preparations.

The mesogloea is homogeneous and destitute of cells. Its ectodermal surface in the disc is raised into thin lamellæ for the support of the longitudinal muscle fibres. These lamellæ do not terminate immediately below the nervous layer, however, but branch, and send branching fibres up through this layer (Fig. 6, *pm.*). This is very clearly seen in some of my preparations, especially some which were stained with eosin. One is reminded by this arrangement of what R. Hertwig ('88) has described as occurring in *Ilyanthopsis longifilis*.

The endoderm is destitute of Zoöxanthellæ. Its cells give rise at their bases to muscle fibres, which, as usual, are arranged circularly. Occasional gland cells are seen, but I did not find them so numerous as the Hertwigs figure them in *C. membranaceus*; they are of one kind only, namely, the granular club-shaped kind.

(b) *The Column Wall.*

The ectoderm of this portion of the body is characterized by the great abundance of large nematocysts, with an irregularly

coiled thread, similar to those originally described by Haime. They are especially abundant in the upper part of the column, and occur throughout the entire thickness of the epithelial layer down to the nerve layer. In the lower part of the epithelium, however, they are principally represented by highly refractive globules of various sizes, some perfectly homogeneous, others split with irregular portions of various sizes; these globules I judge to be developing nematocysts on account of their behavior to various staining reagents, which is exactly like that shown by the fully developed cysts. They have been described and similarly identified by Jourdan. A second form of nematocyst is also present, chiefly in the outer portions of epithelium. It is much smaller than the large *Cnidæ glomiferæ* (Gosse), and is cylindrical, measuring $28\ \mu$ in length, and $5\ \mu$ in breadth. It is well differentiated by both gold and saffranin staining, taking with the former a faint pinkish tinge, and with the latter a bright orange. It is clear, the spiral portion of the filament not being visible, while the "Axenkorper" (Möbius) is very readily seen. A few nematocysts resembling those found in the tentacles and disc also occur. Undoubtedly too much stress has been laid upon slight differences in shape, in distinguishing different forms of nematocysts. Haime certainly erred in this respect, and von Heider also, though to a less degree. The two forms described by the latter from the tentacles differ only in size, and are probably the same, and it does not appear to me to be necessary to distinguish between the two forms of *Cnidæ glomiferæ* he describes from the column wall. Probably the second form I have described above is identical with von Heider's form α from the column epithelium.

The epithelium is covered on its free surface by a cuticle. Gland cells are very abundant, and are of the same kinds as were found on the disc.

The nerve layer consists of a very strong band of fibres, which lies immediately above the muscle band. It is traversed at right angles by fine processes, both from the epithelial cells and from the mesogloæal lamellæ which support the muscle fibres. A few nuclei are to be seen lying among the nerve fibres, but they are very few and small, resembling those found in the nerve layer of the disc. They are probably gan-

glion cells, but there is certainly no such development of ganglion cells as Danielssen describes as occurring in his *C. borealis*.

This description agrees essentially with that of the Hertwigs. It is well known, however, that von Heider's observations differ somewhat from those of the Hertwigs. He describes as occurring in the lower part of the epithelial layer an "Interbasalnetz" of fibres, formed by the anastomosis of fine branching processes of the epithelial cells. In the meshes of the network are fine, sharply outlined points, which are supposed to be cross-sections of delicate fibrils. These fibrils are nervous, and send branches upwards to the epithelial cells, and downwards to the mesogloea. The Hertwigs, in discussing in an appendix von Heider's results, identify this "Interbasalsubstanz" with their nerve band. This, however, is a mistake. In all sections taken from one of my specimens I get an appearance similar to that shown in the Hertwigs' Pl. VIII., Fig. 11; in all the sections taken from another specimen, I get von Heider's "Interbasalnetz." Why there is this difference in the two specimens I cannot say. It may be due to a difference in the amount of contraction. The nerve band in those preparations which show the "Interbasalnetz" is plainly visible, lying between the muscle layer and the network, and corresponding therefore with the fibrillar layer which von Heider describes as appertaining to the "mesoderm," which is shown in his Pl. V., Fig. 35, *f*. It is this fibrillar layer then, and not the "Interbasalnetz," which is the nerve band. It is possible that the "Interbasalnetz" appearance may be produced by the great contraction of the mesogloea and of its processes, which extend up into the epithelial layer. This idea is strengthened by the fact that gland cells and nematocysts are of very frequent occurrence in the network, — a fact that shows that it belongs to the epithelial layer.

The longitudinal musculature of the column is as usual well developed. For some distance below the margin it is no higher than the musculature of the disc, but lower down it increases in size, reaching its greatest height about the middle of the column, where it is many times higher than on the disc. This height it retains almost unaltered to within at least 2 mm. of the posterior extremity, except along the dorsal median line, where it is throughout low as in *C. membranaceus*. It has essentially

the same structure as in the disc, except that the fibres near the base of the lamellæ are much smaller in diameter than those which cover the greater portion of their surface. I have not been able to discover the slightest trace of circular muscles intermingled with the longitudinal, as Danielssen describes in *C. borealis*, Danl.

The mesoglœa presents the same structure here as on the disc. The muscle lamellæ are prolonged at their free edges into numerous fine branching processes, which traverse the nerve layer, and pass up into the epithelial region of the ectoderm. Continuations of the epithelial cells also traverse the nerve layer, and pass down between the muscle lamellæ, and are perhaps nervous, or partly nervous, and partly the basal portions of the "Stützzellen." A circular musculature is present on the inner surface of the mesoglœa.

(c) *The Stomatodæum.*

As already stated, the surface of the stomatodæum is raised into numerous longitudinal folds, each of which corresponds to an interval between two mesenteries (Fig. 7). The ectodermal surface of the mesoglœa is raised into slight ridges corresponding to the folds of the ectoderm, and is provided with short lamellæ supporting the longitudinal muscle fibres, which have a much smaller diameter in this region than elsewhere. The delicate branching processes from the mesoglœal lamellæ are very evident (Fig. 7), especially those which arise from the muscle processes covering the ridges. They can be traced for some distance up into the ectodermal epithelium, forming supports for its cells.

The epithelial and nerve layers have the same structure and histological characters as in *C. membranaceus*. Circular muscles occur in the endoderm.

(d) *The Mesenteries.*

The disc in *Cerianthus* being funnel-shaped, a section made transversely through the column wall in its uppermost part will cut the disc tangentially (though slightly obliquely to its thickness) and the marginal angles of the mesenteries obliquely; that is to say, the section of the mesenteries shows their actual thickness, but it is at an angle to both their length and breadth.

In such sections the mesenteries present a very different structure from what is found lower down.

The endoderm in such a section (Fig. 8) resembles very closely in structure that of the column wall, but lower down, in sections which pass through the column wall and the stomatodæum, it is much lower, very granular, and without any trace of cell outlines. In the gonophoric region (Fig. 9) it again becomes high, higher even than in the uppermost region. The protoplasm of the cells is crowded towards their free extremities, which take the carmine stain, and in this region the nuclei are most abundant; towards the mesogloea, however, the cells form a network (Fig. 10), the meshes of which are mainly occupied by a substance which does not stain. Slight traces of a granular substance are also present, and in some regions there are large numbers of apparently homogeneous spherical bodies (Fig. 10), which do stain somewhat deeply, and which vary considerably in size. It is possible that they may be nuclei, but I am rather inclined to think from their homogeneity and varying size that they are food particles. They occur also in the endoderm of other regions.

It is possible that the network is formed by branching and anastomosing processes of mesogloea, but such an origin for it could not be made out. If it should be of this nature, it would be in accord to a certain extent with what Danielssen has described in his *C. borealis*. Delicate lamellæ having a wavy outline project from the mesogloea of the mesenteries into the epithelium: upon their surface are arranged both longitudinal and transverse muscle fibres. No such arrangement occurs in *C. Americanus*, nor apparently in *C. membranaceus* and *solitarius*, but, as stated, the network, if mesogloæal, might be regarded as representing it. The granular substance which lies along the fibres composing the network no doubt corresponds to the muscle fibres described by Danielssen, these, like the pinnate lamellæ, being "äusserst dünne." Maceration preparations of *C. Americanus* failed to show the presence of muscle fibres in the meshes of the network. In the endoderm of the mesenteries, as elsewhere, no Zoöxanthellæ are present.

In the sections which pass through the disc and column wall, the endodermal musculature of the mesenteries is very clearly seen (Fig. 8). The muscle fibres form a single flat layer on

both surfaces of the mesogloea, and in the sections are cut more or less obliquely, some being cut almost transversely, and others more longitudinally. From the way they run, however, it is clear that they are really transverse, and their apparent oblique, or even longitudinal direction, is due to the manner in which the mesentery is prolonged up into the angle formed by the column wall and the funnel-shaped disc. Lower down they are not so apparent, and in transverse sections of a mesentery in the gonophoric region they cannot be made out with certainty, although maceration preparations show their presence. This arrangement of the muscles agrees with what the Hertwigs have described for *C. membranaceus*.

The mesogloea is differently developed in different regions. It is thinnest in the stomatodæal region, and somewhat thicker in the gonophoric region, being in both these parts almost homogeneous, and without any cells in its substance. In the uppermost angle of the mesenteries it is much thicker than elsewhere (Fig. 8), and contains cavities, reminding one of the cavities found in the mesenteries of the Zoanthæ, except that the contents are not cellular as in that group, but consist of a granular substance which does not stain at all with borax carmine.

As stated above, all my specimens were female. The ova are large, and are imbedded in the mesogloea of the mesenteries, as is well shown in the preparation figured (Fig. 9), where the mesogloéal investment has separated from the ovum at one point. The nucleus is large, and is always eccentric, usually projecting very noticeably beyond the general surface of the ovum, which is packed with densely staining yolk granules. One large nucleolus is always present, but the rest of the nuclear substance in all my preparations is apparently broken down, the nucleus appearing as an irregularly shaped space with well-marked walls, containing the large nucleolus and a few granules.

The histological details of the mesenterial filaments I hope to describe fully in a future paper.

I have not been able to find any mesenterial stomata in *C. Americanus*.

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March 11, 1890.

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EXPLANATION OF PLATES VI. AND VII.

PLATE VI.

FIGS. 1 and 2. *Cerianthus Americanus*, Ag. — Fig. 1, natural size; Fig. 2, reduced one-third.

PLATE VII.

FIG. 1. View of specimen laid open by a longitudinal incision passing near the mid-dorsal line. *si* = siphonoglyphe, *ac* = acontia, *st* = stomatodæum, 3 = mesentery of third grade.

FIG. 2. Semi-diagrammatic, showing the relations of the mesenteries of the different grades. 3 = mesentery of third grade, 4 = mesentery of fourth grade.

FIG. 3. Section through ventral portion of column, just above the lower end of the stomatodæum. *D* = ventral directive mesenteries, 1 and 1' = mesenteries of the first grade, 2 = mesentery of the second grade, 3 and 3' = mesenteries of the third grade, 4, 4', 4'', and 4''' = mesenteries of the fourth grade.

FIG. 4. Section through ventral portion of column wall, about 2 mm. below Fig. 3.

FIG. 5. Section through ventral portion of column wall, about 1.5 mm. below Fig. 4.

FIG. 6. Portion of tangential section of disc. *cm* = circular muscles, *mg* = mesogloea, *lm* = longitudinal muscles, *n* = nerve layer, *pm* = process of mesogloea. (Zeiss obj. J, oc. 2.)

FIG. 7. Transverse section of stomatodæum (Zeiss obj. J, oc. 2).

FIG. 8. Section of mesentery in the angle formed by the meeting of the disc and column wall (Zeiss obj. J, oc. 2).

FIG. 9. Transverse section of mesentery in the gonophoric region, with ovum (Zeiss obj. D, oc. 2).

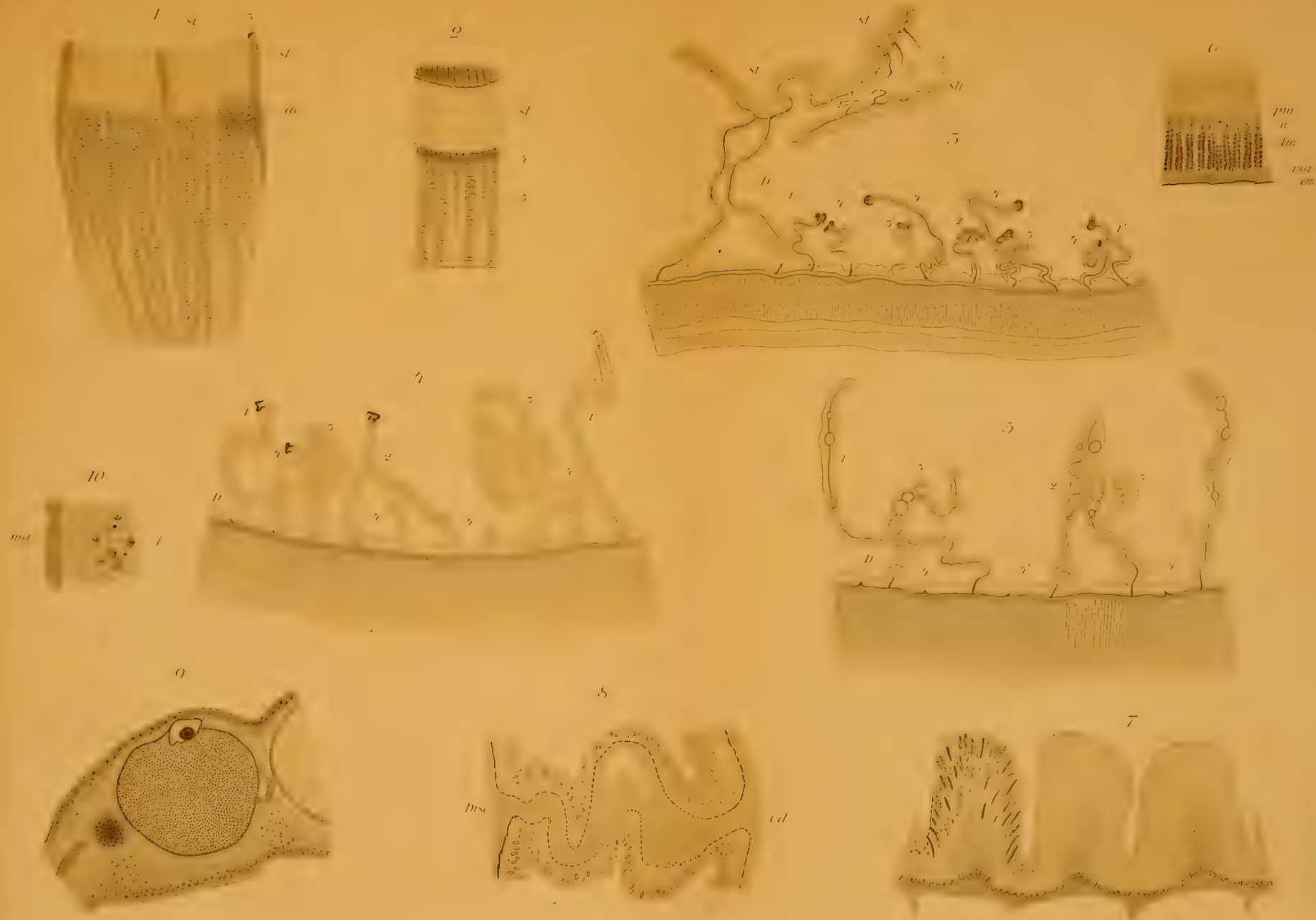
FIG. 10. Portion of Fig. 9, more highly magnified to show the network. *mg* = mesogloea, *f* = food particle (?). (Zeiss obj. J, oc. 2.)

Fig. 1.



Fig. 2.





ON THE GUSTATORY ORGANS OF SOME OF THE MAMMALIA.

FREDERICK TUCKERMAN.

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THE researches which are recorded in the following pages were carried on in the Biological Laboratory of Clark University, Worcester, during the winter of 1889-90.

I am greatly indebted to the University for kindly obtaining much of the material upon which this paper is based. I also desire to express my thanks to Mr. Agassiz, Curator of the Museum of Comparative Zoölogy at Cambridge, Dr. Whitman, Professor of Animal Morphology in Clark University, Mr. Bumpus, Associate Professor of Biology in Brown University, and Dr. J. N. Hall, of Sterling, Colorado, for their kindness in supplying me from time to time with valuable specimens. The greater part of the material had been kept in dilute or ordinary spirit. Of the remaining specimens, some were hardened in absolute alcohol, others in osmic acid, and still others in Müller's fluid, either alone or in a mixture of Müller's fluid and alcohol in various proportions. The parts of the specimens intended for microscopical examination were embedded in celloidin, and for staining the tissues the usual reagents were employed.

A table containing some comparative data respecting the gustatory structures of the forms considered in the following pages is appended to the paper.

THE TONGUE OF *Didelphys virginiana*.

I examined several tongues of *Didelphys*, most of them from adult animals. All of the specimens had been hardened in spirit before I received them.

General Description.—The organ measures 75 mm. in length, 18.5 mm. in breadth, and 13 mm. in thickness. Anteriorly it is free from the frænum linguæ for 34 mm. The under surface is smooth and marked by a longitudinal median ridge extending from the frænum to the tip. On either side of the ridge is a wide but shallow groove. The tip, which is somewhat obtuse, is bordered by a delicate fringe of simple filiform papillæ, the latter being probably tactile in function. The upper surface is impressed by a number of transverse furrows, corresponding to the roof of the palate. Anteriorly it is covered with closely packed compound filiform papillæ, to be presently described. The fungiform papillæ are of fair size, and appear to be normal in structure. They are not, however, very numerous, and, as

usual, are smallest about the tip. The circumvallate papillæ are three in number. They are arranged in an isosceles (or more rarely an equilateral) triangle, the base of which measures 4.6 mm. The apex of the triangle is turned towards the epiglottis, and is distant some 12 mm. from the base of the tongue. The posterior papilla is situated directly in the middle line of the organ, and is much larger than the anterior papillæ. The region behind the triangle formed by the circumvallate papillæ lacks the usual fleshy elevations, and is quite smooth; the immediate area around the papillæ, however, is more or less papillate. The lateral organs of taste (papillæ foliatæ) were so effectually concealed that the superficial examination of the tongue failed to reveal their presence. At each side of the tongue, just above the line of union of the upper and lower surfaces, is a fringe of rather coarse filiform papillæ, curving upwards, inwards, and backwards. The fringe terminates before reaching the base of the glosso-palatine arch.

The tongue of a young *Didelphys* presented some points of interest. This organ was 19 mm. in length and 7 mm. in breadth. The dorsum was marked anteriorly by a longitudinal mesial raphé. The raphé disappeared about 12 mm. from the tip. The tip was rounded, and fringed with simple papillæ as in the adult organ. The circumvallate papillæ were arranged in the usual triangle, but were very closely set and very near the base of the tongue. The lateral fringe of filiform papillæ at the back of the tongue was well marked.

The Mechanical Papillæ.—The general surface of the tongue, anterior to the triangle formed by the circumvallate papillæ, is covered with compound filiform papillæ. Interspersed among them are a lesser number of filiform papillæ of a simple type. About the tip, where the papillæ are smaller and more closely set, there appear to be about thirty to the square millimetre of surface. Posteriorly there are only half that number covering the same area. Each papilla rests upon one or more papillary upgrowths of the mucosa, and, at a short distance from its base, breaks up into a varying number of very long, slender, recurved secondary papillæ or processes. The number of these processes varies greatly even in the same region. There are seldom less than ten, and I have seen more than fifty. The average number appears to vary from sixteen to twenty. The free portion

frequently exceeds a millimetre in length, the transverse diameter being only about 0.021 mm. Anteriorly the papillæ are longer and more delicate than elsewhere. As they near the tip, however, they decrease in number and are somewhat shorter. Posteriorly they frequently bear a recurved spine (doubtless a transitional form) in addition to their more slender secondary processes. Still further back, in some papillæ, the secondary processes appear to have coalesced, forming a single stout, sharp-pointed, hook-like spine, as in many of the higher Mammalia. Papillæ thus modified are also sparingly interspersed among the others at various parts of the dorsum. The secondary papillæ form one or more incomplete rings at the summit of the main upgrowth, leaving within a horseshoe-shaped cavity. Where two rings exist, the inner one is usually less complete than the outer. More rarely, within the inner ring a few secondary papillæ are irregularly scattered. The secondary processes separate from the papillary body first in front, those of the posterior side being given off at a higher level. A few single filiform papillæ, somewhat hair-like, are scattered over the dorsum, particularly its anterior part.

When lowly magnified, the main body of the papilla (in vertical section) appears to consist largely of a mass of cells in the form of a thick column or inverted cone. This mass is composed of several distinct but not always sharply marked layers. The basal layer is quite thin, and consists of small but clearly defined, deeply staining columnar cells. Succeeding this is a very thick layer, consisting below of nucleated polyhedral cells, and above of fusiform cells. The cells of this layer are largely granular and stain readily in hæmatoxylin, but less deeply than those of the layer underlying. Resting upon this layer, and also covering the lateral area of the papilla, is one consisting of elongated cells, more or less granular. These cells are somewhat attenuated below, and large and swollen above and at the sides. The nuclei are not always readily distinguishable, but are of unusual size. The cells are not stained by hæmatoxylin. Against this layer is a comparatively thin one, likewise composed of cells fusiform in shape. The cells stain deeply, and are apparently of the same general character as those constituting the bulk of the secondary papillæ. The entire free portion of the secondary processes in the anterior dorsal region

stains quite deeply in hæmatoxylin. The secondary papillæ of the middle dorsal region, in which the cornification of the epithelium is further advanced, are much less affected by staining reagents. Externally the papillæ are covered by a thin layer of ordinary stratified epithelium.

This type of compound filiform papillæ is characteristic of the marsupial tongue, if not peculiar to it. The papillæ, as suggested by Poulton, who first described them,¹ have doubtless been modified in a special manner for the capture of small insects. The same observer has suggested the term "coronate" for those papillæ which are surmounted by a ring of recurved hair-like processes, and the term "fasciculate" for those in which the appearance is more brush-like. For the compound filiform papillæ of *Didelphys virginiana* the latter term would be more closely descriptive than the former.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ.—The posterior papilla follows quite closely the type common to the higher animals. The anterior pair resemble the corresponding papillæ of *Belideus* and *Phalangista*, but are further advanced. The posterior papilla measures 0.75 mm. at its widest part, and is 0.70 mm. in height. The upper surface is somewhat uneven, and overtops slightly the adjacent lingual area. The trench encircling the papilla is narrow and fairly uniform in breadth. The anterior papillæ are somewhat elongated, and average 0.60 mm. in breadth and 0.90 mm. in height. They are deeply set, their summits barely reaching the level of the general lingual surface. Serous glands are abundant beneath and at the sides of the papillæ, and their ducts open into the trenches at their base and lower part.

The taste-bulbs of the posterior papilla are mainly confined to the inferior half of the lateral area. They are disposed in a zone of twelve to fourteen closely packed tiers. The bulbs of the anterior papillæ are similarly arranged, though with an increased number of tiers. There are about sixty-five bulbs in a tier. The bulbs of both regions are fairly uniform in size and shape, and measure 0.054 mm. in length and 0.034 mm. in breadth.

¹ "On the Tongues of the Marsupialia," *Proc. Zool. Soc.*, 1883.

The Lateral Gustatory Organs. — These organs are somewhat rudimentary, and consist of five or six irregular folds of the mucous membrane. The intervening furrows are narrow, and vary in depth from 0.5 mm. to 1 mm. Serous glands are fairly abundant, and their ducts discharge at the bottom of the furrows. Sections through some regions of the organ show the sides of the folds filled with bulbs, there being twenty or more tiers of them. As a rule, however, they are fewer in number and restricted to the lower half of the lateral area. They measure 0.046 mm. in length and 0.030 mm. in breadth.

Bulbs were present at the upper part of the fungiform papillæ. In some sections as many as five could be counted, but they were for the most part simple in structure, their apices failing to reach the superficial layers of the epithelium. They are very small, averaging only about 0.030 mm. in length and 0.016 mm. in breadth.

In the young *Didelphys* the papillæ and trenches, save in a few instances, were undifferentiated. The glands and their ducts were likewise incomplete in their development. The bulbs were mainly epithelial in position and, as usual in embryos and the new-born, had only developed at the upper part of the papillæ, the lateral area being destitute of them. One of the more advanced among them measured 0.027 mm. in length and 0.018 mm. in breadth. I failed to identify bulbs in the epiglottis of *Didelphys*.

THE TONGUE OF *Bettongia cuniculus*?

This was the tongue of an embryo. It had been kept in spirit.

General Description. — The organ is long and narrow, and is free for nearly half its length. The under surface is marked by a longitudinal median ridge extending from the frænum to the tip, and the papillate surface is impressed anteriorly by a mesial raphé. Fungiform papillæ are freely distributed over the dorsum. The circumvallate papillæ form a triangle near the base of the organ. The anterior papillæ could only be distinguished with difficulty, their apices being barely visible within the narrow, slit-like openings of the trenches. The posterior papilla, however, was clearly defined.

There are some indications of bulbs in the epithelium and

mucosa of the upper part and sides of the anterior papillæ. In the posterior papilla there are three or four tiers of bulbs. They rest in depressions of the mucosa, and their apices traverse the epithelium, but fail to penetrate its outer layers. They also occur to some extent on the upper surface of the papilla. Serous glands and ducts are present in the mucosa and submucosa, the latter being very plentiful. They open into the trenches at their deeper part. No bulbs were detected either in the fungiform papillæ or epiglottis. No search was made for the lateral gustatory organs.

THE TONGUE OF *Phascolomys wombat*.

This tongue (that of a young animal) had been kept in dilute spirit, and was not in a very favorable condition for minute examination. The hardening was completed in absolute alcohol.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ.—The papillæ are arranged in the usual triangle, the sides being a little shorter than the base. Their summits are circular, or nearly so, and they are of nearly equal size. They measure 0.75 mm. transversely, their height being somewhat less. At their upper part they bear many secondary papillæ; below they are attached to the tongue by a narrow pedicel. The trenches are narrow and uniform in width. Serous glands are abundant, and their ducts discharge at the usual places.

The taste-bulbs are mainly restricted to the lower half of the lateral walls of the papillæ, although isolated ones not infrequently occur at higher levels. They are disposed in about ten tiers, each tier containing on the average seventy closely packed bulbs. They are also present in the epithelium of the outer wall of the trench. Here they are disposed in five tiers. The bulbs are long and narrow, and present considerable variation in size. The mean length varies from 0.060 to 0.075 mm., and the mean breadth from 0.024 to 0.035 mm. In a transverse section through the centre of a bulb (0.030 mm. in diameter), I counted some twenty-seven cells. In the subepithelial tissue beneath the bulb region of the papillæ is a rich nervous network. From this network fibrils run to the bases of the bulbs

and also pass between them. Large ganglion cells are likewise present in the axes of the papillæ. For lack of sufficient material no search was made for the lateral gustatory organs, though they are doubtless present.

The fungiform papillæ are quite numerous, especially over the anterior dorsal surface. They are of the usual type and probably contain bulbs, although none were found. No bulbs were detected in the epiglottis.

THE TONGUE OF *Phascolarctos cinereus*.

This specimen was also from a young animal, and had likewise been kept in dilute spirit. It was subsequently placed in absolute alcohol. For a careful study of this tongue, or that of *Phascolomys*, fresh material, or material in a better state of preservation than that which I had at my disposal, will be necessary.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ.—The papillæ are very clearly defined. They form a nearly equilateral triangle, the base being a trifle less than the sides. The posterior papilla is much the largest of the three, and is encircled by a narrow trench. It is freely movable, and measures 0.55 mm. in diameter and 0.50 mm. in height. Striated muscle-fibres terminate at the base of the papilla. The anterior papillæ are elongated, and measure 0.50 mm. in height and 0.35 mm. transversely. They are set in deep trenches, which open on the surface with narrow, slit-like apertures. Anteriorly their summits terminate in an apex. Further back they lose their apex, and become more convex. At their extreme posterior limits they are completely roofed over. Their bases are somewhat constricted, and their apices do not reach the level of the opening of the trench. Serous glands are plentifully distributed to this gustatory area, their ducts opening at the bottom of the trenches. Nerves and large ganglion cells are present in the anterior papillæ.

The bulbs are quite numerous. The arrangement of those of the posterior papilla is much the same as in the circumvallate papillæ of *Phascolomys*. In the anterior papillæ there appear to be upwards of twenty-five tiers, a well-filled tier containing

about seventy-five bulbs. The apex of the papilla is free from bulbs, but the ridge behind it bears them over the whole of its circumference. The bulbs were too indistinct for their finer details to be studied.

Papillæ of the fungiform type are not very abundant, but appear to be of normal structure. They are largest on the dorsum and sides, just in front of the circumvallate papillæ, and contain bulbs. The usual fringe of filiform papillæ at the side of the base of the tongue, just above the lateral organ of taste, was present. Below the fringe were a few small, mound-like elevations, which doubtless marked the seat of the lateral taste organ. Similar elevations are present in *Halmaturus* and other marsupials. Unfortunately, the material was not in a condition for closer examination. The epiglottis was not examined.

THE TONGUE OF *Dasypus peba*.

The material consisted of two spirit specimens, — a whole tongue and the back part of a smaller one.

General Description. — The tongue is long and narrow, and tapers gradually to a point. It measures 51 mm. in length and near the base is 10 mm. in diameter. It is free for 34 mm. from the frænum, two-thirds of its entire length, and is thus capable of great prehensile power. The under surface is finely wrinkled, and marked by a median ridge extending from the frænum to the tip. The ridge is impressed longitudinally by two parallel grooves. It is likewise transversely furrowed, the furrows being parallel and 1.5 mm. apart. The anterior two-thirds of the papillate surface is sheathed with a thick layer of partially cornified epithelium. The posterior third is transversely wrinkled, the furrows being parallel and running across the tongue. The basal end of the organ bends somewhat abruptly downwards, forming a kind of pit. From this pit a deep groove extends along the middle of the dorsum for 6 mm. Fungiform papillæ are not numerous, but are quite evenly distributed over the middle and anterior dorsal surface and upon the sides. Some of the papillæ appear to be set in furrows, with their summits below the level of the lingual surface. The circumvallate papillæ are two in number. They are 5 mm. apart, and 15 mm. from the base of the organ. I failed to find the lateral organs

of taste in this species, but I think there can be but little doubt of their existence.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ.—The papillæ are 0.40 mm. in diameter and 0.95 mm. in height. Their upper surfaces are more or less flattened, and their sides are vertical, or nearly so. The trenches encircling them are narrow and very deep. Serous glands are but sparingly present, and their ducts open at or near the bottom of the trenches. At the lower part of the papillary axis are a few isolated ganglion cells. The bulbs are disposed on the lateral wall, often nearly filling it. They also occur to some extent on the free surface of the papillæ. The average number of tiers appears to be about eighteen (though there may be twice that number), a well-filled tier containing from sixty to seventy bulbs. The bulbs show some indications of a neck. They measure 0.054 mm. in length, their greatest transverse diameter being 0.030 mm. No bulbs were detected in the fungiform papillæ or in the epiglottis.

THE TONGUE OF *Dasypus villosus*.

I received three specimens of this tongue. They had been kept in spirit, and the tissues were in a fair state of preservation.

General Description.—The organ is long and narrow, and tapers gradually to a point. It measures 81 mm. in length, and is perfectly free for 50 mm. from the frænum. At its posterior part it is 12 mm. in breadth and 14 mm. in thickness. The under surface is marked by a distinct median ridge leading from the frænum to the tip. The extreme basal portion of the organ is bent somewhat abruptly downwards, as in *Dasypus peba*. The upper surface is quite densely papillate over nearly its entire area. Papillæ of the fungiform type are sparingly scattered over the dorsum and sides of the tongue. The two circumvallate papillæ are on the same transverse line, and are set quite close to the lateral margins of the organ. They are 7.3 mm. apart, and 17 mm. from the base of the tongue. There is a lateral gustatory organ at each side of the base. The organs are marked externally by three or four small, irregular openings, running transversely to the long diameter of the tongue.

The Mechanical Papillæ.—These papillæ differ to some extent from the compound filiform papillæ of the marsupial tongue. The secondary papillæ are fewer in number, there being not more than five or six to a papilla as a rule, and resemble somewhat the stout, hard spines of the Carnivora. Many of the papillæ bear at each lateral border a single recurved spine, the space between being packed with epithelium. When viewed in horizontal section they present a horseshoe-shaped cavity. These papillæ may be looked upon as representing an intermediate type between the “coronate” and “fasciculate” papillæ of the Marsupialia and the corresponding papillæ of still higher forms. Another and more simple form of papilla occurs on this tongue near the lateral margins. It consists of a single papillary upgrowth of the mucosa, overspreading which is a layer of stratified epithelium. From the bed of epithelium rises a single sharply pointed spine. The spine is cornified at its upper part, and directed inwards and backwards.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ.—The papillæ were not developed alike in all the specimens. While some of them resemble the papillæ of higher animals, others approach more nearly the marsupial type. The former, or more recent type, are 1.3 mm. in diameter and 1.1 mm. in height. They are flattened on top, and barely reach the level of the lingual surface. The bulbs are disposed around the lower part of the lateral area in eighteen closely packed tiers. The circumvallate papillæ of less recent type are taller than the foregoing, and their sides converge as they approach the opening of the trench. Their lateral area is filled with bulbs to within a short distance of the top, there being often thirty tiers of them. They measure 0.051 mm. in length and 0.030 mm. in breadth. Serous glands are quite abundant, the ducts opening into the trenches at their base and sides.

The Lateral Gustatory Organs.—The lateral gustatory organ of *Dasypus villosus* is not unlike that of *Procyon lotor* (described by the writer in the “Journal of Anatomy,” Vol. XXIV., 1890). The superficial examination of this region showed several irregular slit-like openings, but I only succeeded in obtaining sections through one of them. This opening was 0.20 mm. in

width, and led into a large, irregular shaped cavity or recess, 0.55 mm. in depth and 1 mm. in diameter. The walls of the recess are not very thick and are lined with stratified epithelium, resembling in the main that of the adjacent lingual surface. From the floor of the recess rise two ridges, which largely fill it. The larger of the two measures 0.40 mm. in diameter and 0.45 mm. in height. Serous glands are fairly abundant, and their ducts open into the spaces between the ridges, and also at the sides of the recess towards its deeper part. The ridges bear bulbs over their entire circumference. Bulbs are also very irregularly scattered in the walls of the cavity, and likewise occur in clusters near its mouth. They are small, measuring 0.042 mm. in length and 0.024 mm. in breadth.

The fungiform papillæ appeared to be of the usual mammalian type, but were destitute of bulbs. The latter were likewise wanting in the epiglottis.

THE TONGUE OF *Dasypus minutus*.

This specimen had been kept in spirit, but was not in a favorable condition for minute study.

General Description.—The organ is 33 mm. in length, 5.5 mm. in breadth, and is free from the frænum for 14 mm. The under surface has the usual ridge, the upper anterior region being grooved transversely. The tip was somewhat less pointed than in the other Edentata examined. Although the entire tongue was cut into sections, no circumvallate papillæ or lateral gustatory organs were found. It is quite probable, however, that they were overlooked. The fungiform papillæ are of good size, and contained bulbs. In a single section of one of them I counted no less than six. They measured on the average 0.039 mm. in length and 0.021 mm. in breadth. Simple and compound filiform papillæ were present, the former being interspersed among the latter. The epiglottis was not examined.

THE TONGUE OF *Chlamyphorus truncatus*.

I received only the anterior four-fifths of this tongue. The piece measured 36 mm. in length. It had been kept in spirit, and was sufficiently firm for cutting.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ. — The two papillæ required the aid of a powerful lens to reveal their presence. They are as usual on the same transverse line, 1.7 mm. apart, and lie completely concealed in deep and narrow trenches, their apices, which are inclined inwards towards the median line, being slightly below the openings of the latter. At a short distance above their bases (which are constricted) the papillæ measure 0.23 mm. in diameter, their height being 0.6 mm., or nearly three times the transverse diameter. I do not think it probable that the mouths of the trenches can be closed. The arrangement of the muscles beneath the papillæ suggests a possible drawing downwards of the entire region, but not of the papillæ alone. Glands of the serous type are sparingly scattered through the connective tissue stroma underlying the papillæ, and their ducts open into the trenches at various levels.

The bulbs are restricted to the lower two-thirds of the lateral area of the papillæ. There may be seventeen or more tiers of them. They were not clearly enough defined for me to determine the mean dimensions of the typical bulb of this species. One which I measured, and which was probably somewhat below the mean, was 0.030 mm. in length and 0.018 mm. in breadth. No lateral organs of taste were found on this piece of tongue.

The circumvallate papillæ of *Chlamyphorus* approach quite closely the marsupial type, the resemblance between them and the anterior papillæ of *Belideus* and *Phalangista* being very marked.

THE TONGUE OF *Lepus campestris*.

I received a fresh specimen of this tongue. The organ was placed in a mixture of five parts Müller's fluid and one part alcohol. After remaining in this mixture for fourteen days, it was washed for a few hours in running water, and then transferred to ordinary spirit, where the hardening was completed.

General Description. — In many rodents the posterior portion of the tongue rises somewhat abruptly above the level of the anterior. This is a characteristic feature of the tongue of *Lepus*. The organ shows two well-marked divisions, a more or less flattened and expanded anterior portion, and a raised pos-

terior part. The two divisions are of nearly equal length, the total length of the organ being 55 mm. The anterior division is 15 mm. in breadth, 10 mm. in thickness, and is free from the floor of the mouth for 14 mm. The upper surface and sides of this division are covered with small, densely packed, cone-shaped papillæ, the apices of which are directed backwards. The epithelium sheathing the papillæ is dense and imbricated, and in their upper half either partly or wholly cornified. The papillate surface is impressed by a mesial furrow, extending from the anterior limits of the posterior division nearly to the tip. The tip is short, broad, and obtuse. The under surface is somewhat wrinkled, and marked by a longitudinal median ridge. Fungiform papillæ are not especially numerous. They are rather sparingly distributed over the anterior dorsal surface, and the posterior division of the organ appears to be nearly destitute of them. About the tip, however, particularly over its inferior part, they are of good size and fairly abundant. The posterior division, which rises somewhat abruptly above the level of the preceding, is 13 mm. in breadth, and about the same in thickness. The upper surface is somewhat convex, and, in front of the circumvallate area, is covered with closely set mechanical papillæ, the points of which are directed backwards. The two circumvallate papillæ are placed one on either side of the median line, and are 6 mm. apart. The lateral gustatory organs (papillæ foliatæ) are situated obliquely on each side of the back of the tongue, anterior to the glosso-palatine arch and circumvallate papillæ, their anterior extremity being directed downwards and inwards. They are 5 mm. long, and, at their anterior end, 3 mm. in breadth. Viewed from above, they are small, convex elevations, their lateral contours converging and forming an apex at the posterior limits of the organ.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ. — The general surface in front of the papillæ is covered with small papillary elevations. The immediate area around them, however, is unapillate. They measure 0.60 mm. in diameter, and are a trifle less in height. They are flattened on top, and the trenches which encircle them are narrow and of uniform width. Mucous glands are very plentiful, more so than those of the serous type. The ducts of

the former traverse the mucous membrane, and open, somewhat obliquely, on the free lingual surface; while those of the latter discharge into the trenches at their lower part. The mucosa forming the body of the circumvallate papillæ consists of three main portions or lamellæ. The central lamella is much the largest, and overtops the other two. At its upper part it is cleft into a number of secondary papillæ, the spaces between which are filled to a common level with stratified epithelium. The depressions between the lamellæ not infrequently dip down nearly to the base of the papillæ, but they are for the most part filled by epithelium. The average thickness of the stratified pavement epithelium covering the papillæ is about 0.045 mm. This layer is thicker above than at the sides, but the difference is only slight.

The bulbs of this gustatory area fill the middle third of the papillary wall. Those of the outer wall of the trench are somewhat similarly placed. The bulbs, to all appearance, are in contact by their edges, and, in the papilla, are disposed in a zone of five tiers. Those embedded in the epithelium of the outer wall are arranged in a girdle of four tiers. From horizontal sections I estimated the mean number of bulbs in a tier of the papilla at sixty, the mean number in a tier of the outer wall being about seventy-five. The bulbs vary somewhat in size and shape, but most of them have a fairly well-developed neck. Their mean length is 0.049 mm. and their mean breadth 0.030 mm., they being a little smaller than in their eastern congener, *Lepus americanus*. The papillæ are well supplied with nerves. Medullated fibres of the glosso-pharyngeus enter the papillæ at their base, and their finer (non-medullated) branches form a delicate network in the mucosa directly beneath the bulb region. From the network fibrils enter the bulbs, and also pass between them into the epithelium.

The Lateral Gustatory Organs.—The superficial dimensions of these papillæ have already been given. Each foliate structure consists of fourteen folds, nearly all of which bear bulbs on their lateral area. The folds are separated by narrow furrows, having an average depth of 0.30 mm. The mucosa composing the body of each fold is divided, as is usual in *Lepus*, into three secondary folds or lamellæ, the primary or central lamella being taller and slightly broader than the two lateral.

Spread over the lamellæ is a thin layer of stratified pavement epithelium, which usually fills up the depressions between them to one level. Serous glands are plentiful in this region of the tongue, and their ducts, which are exceedingly numerous, open at the bottom of the furrows. The bulbs are disposed, midway between the superior and inferior limits of the lateral wall of the folds, in four to six closely set tiers. Each tier contains about sixty bulbs in its entire length. I have counted as many as eighty in a tier, but this number is largely in excess of the mean. The dimensions of the bulbs are the same as in the circumvallate papilla. The fungiform papillæ, particularly those of the tip of the tongue, contain bulbs. I have not infrequently seen six in a single section of a papilla of this type. The epiglottis was not examined, but bulbs have frequently been found on the posterior surface of this organ in *Leporidae*, not only by the present writer, but by Krause, Davis, and others.

THE TONGUE OF *Geomys bursarius*.

The material consisted of a single spirit specimen.

General Description. — The organ is compressed laterally to fit the narrow mouth cavity, and is somewhat humped up anteriorly and posteriorly. It measures 26 mm. in length, 6.6 mm. in breadth, and 9.5 mm. in thickness, and is quite free for 13 mm. from the frænum. The tip is drawn out into a rounded point. The dorsal surface is covered with recurved filiform papillæ. A shallow median groove runs along the under surface. Fungiform papillæ are not numerous, but occur upon the lower as well as upper surface of the organ. There exists but one papilla of the circumvallate type. It is set directly in the median line, and very near the base of the tongue. The trench, both anteriorly and posteriorly, is incomplete. It is anteriorly incomplete in *Fiber zibethicus*. The lateral gustatory organs lie below the convex surface of the posterior elevated portion, and somewhat anterior to the circumvallate papilla.

GUSTATORY STRUCTURES.

The Circumvallate Papilla. — The papilla measures 0.60 mm. transversely, and is 0.35 mm. in height. At its upper part it bears many secondary papillæ, the depressions between them being filled by the epithelium. Serous glands are plentiful, and

their ducts open at the bottom of the trench. The bulbs are more numerous than in *Fiber*, but are similarly arranged. There are eight tiers of them in the papilla and seven on the outer wall. They form an unbroken chain around the bottom of the trench, which is continued on to both walls to about the same level. Isolated bulbs occur on the free surface of the papilla, and occasionally extend along the outer wall of the trench nearly to its upper angle. The mean length of the bulbs is 0.036 mm. and the mean breadth 0.024 mm.

The Lateral Gustatory Organs. — The lateral taste organs are simple in construction, and resemble those of *Fiber zibethicus*. They consist of four or five unequal folds of the mucosa, separated by furrows of varying depth. The average depth is about 0.20 mm. Serous glands are not abundant. There are three or four tiers of bulbs, forming a chain around the bottom of the furrow. Isolated bulbs also occur near the top of the lateral wall. The bulbs of both the circumvallate and foliate taste areas vary greatly in size and shape. Here they measure 0.036 mm. in length and 0.021 mm. in breadth.

The fungiform papillæ are of normal structure, and bear a few bulbs at their upper part. Those of the papillæ of the mid-dorsal region are only 0.020 mm. in length and 0.010 mm. in breadth. In the papillæ near the tip they are larger, and measure 0.039 mm. in length and 0.021 mm. in breadth. A careful search of the epiglottis and other parts of the larynx failed to reveal the presence of bulbs.

THE TONGUE OF *Hesperomys leucopus*.

This tongue, like the last, was a spirit specimen.

General Description. — The shape of the organ suggests a division into an anterior and a raised posterior part. The posterior division is the longer of the two by one millimetre, the total length of the organ being 13 mm. The anterior division is free from the floor of the mouth for 4.5 mm. The tongue is 4 mm. in breadth and about the same in thickness. The upper surface is covered with small conical papillæ, the apices of which are directed backwards. Anteriorly it is marked by a deep mesial groove which passes through the tip, and is continued for a short distance on to the under surface. The under surface is smooth, and somewhat hollowed out. Fungiform papillæ are

not numerous, but are of the usual type. There is a single, deeply set, circumvallate papilla situated in the median line, 2.5 mm. from the base of the organ. As in *Fiber zibethicus*, the trench is anteriorly incomplete, but perfectly so posteriorly and laterally.

GUSTATORY STRUCTURES.

The Circumvallate Papilla. — The general surface adjacent to this gustatory area is more or less marked by papillary elevation of the mucous membrane. The papilla is quite simple in structure, and measures 0.25 mm. in diameter and about the same in height. It is flattened on top, and nearly encircled by a wide trench. Mucous glands do not seem to be as abundant as usual in this region of the tongue. Their ducts, however, are very large, and pass through the mucous membrane to open on the free lingual surface. Serous glands, on the contrary, are quite plentiful, and even extend into the papilla itself. Their ducts discharge into the trench at its deeper part. The bulbs fill a portion of the lower part of the lateral area of the papilla, but do not always extend to its base. The corresponding region of the outer wall also contains bulbs. They are far from numerous in this gustatory area, there being but three tiers in the papilla and but two on the outer wall of the trench. They traverse the epithelium obliquely, their bases being bent more or less downwards. They have a short neck, and measure 0.048 mm. in length and 0.024 mm. in breadth.

The Lateral Gustatory Organs. — These organs were so inconspicuous as to be overlooked in the superficial examination of the tongue. They are very simple and primitive in character, and resemble in some degree a circumvallate papilla. They consist merely of a single fold of the mucosa, 0.16 mm. in diameter, on either side of which is a wide furrow, 0.18 mm. in depth. In the underlying stroma serous glands are relatively plentiful. There are two or three tiers of bulbs on the walls of the folds, and one or two tiers on the opposed walls of the furrows. They measure 0.042 mm. in length and 0.024 mm. in breadth.

The fungiform papillæ are small, measuring only 0.14 mm. in height and 0.05 mm. in diameter. They usually contain bulbs. The latter lie partly in the epithelium and partly in the mucosa, their apices, apparently, not piercing the outer layers of the

epithelium. They are much elongated, measuring 0.045 mm. in length and 0.018 mm. in breadth. The filiform papillæ follow the usual type. The epiglottis was not examined.

THE TONGUE OF *Castor fiber*.

I received a fresh specimen of this tongue. The organ showed some indications of malformation, notably of the left side. The hardening was conducted as in *Lepus*.

General Description. — The organ measures 74 mm. in length and 25 mm. in breadth. The free portion is short, being only about 18 mm. in length. The upper surface is soft and velvety to the touch. Anteriorly it is flat and somewhat expanded, and terminates in an obtuse apex. The fungiform papillæ are of fair size, and are quite numerous over the anterior fourth of the dorsal surface and tip. Posteriorly they are less abundant, but very conspicuous, projecting somewhat above the lingual surface. Some of those just in front of the gustatory area are very like circumvallate papillæ. At the posterior part of the dorsum, and well towards the base, are three papillæ of the circumvallate type. They form a triangle, the apex of which looks towards the epiglottis. The base of the triangle is 8 mm. in length, the length of the sides being 5 mm. The posterior papilla is 11 mm. distant from the base of the organ. The lateral gustatory structures are situated a trifle anterior to the triangle formed by the circumvallate papillæ, the left lateral organ being much further forward than the right.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ. — The papillæ are 0.26 mm. in diameter and 0.16 mm. in height. Their summits are circular or slightly oval, and are more or less marked by verrucose elevations. Superimposed on one of the papillæ was one of a fungiform type. This is not a very unusual occurrence; I have observed it in *Lepus*, and Schwalbe has called attention to it in *Sus*. A papilla thus placed does not always bear bulbs, and in this instance I could not be sure of their presence. The trenches encircling the papillæ are wide at the mouth, but narrow and uniform at their lower part. Mucous and serous glands are quite abundant in this region. The

ducts of the former open as usual on the free lingual surface, while those of the latter discharge into the trenches at their base and deeper part. Bulbs are not very numerous in the papillæ of this taste area, and are chiefly limited to the lower part of the lateral wall, where they are disposed in five or six tiers. One of the bulbs divided transversely to its longitudinal axis (but probably not exactly at right angles to the true axis) consisted of at least thirty-six cells. The bulbs do not form a continuous zone, but are somewhat irregularly scattered round the edge of the papillary wall. They vary greatly in size and shape, one of the largest measuring 0.060 mm. in length and 0.039 mm. in breadth. The mean length is probably 0.055 mm., and the mean breadth 0.031 mm. A few scattered bulbs are present in the epithelium of the outer wall of the trench where it curves beneath the papilla, and it is not unlikely that they may also occur at higher levels, although none were detected.

The Lateral Gustatory Organs. — I am not quite certain that I had the foliate structure entire. The part in my possession measured 5 mm. in length, and consisted of nine or ten folds, the majority of which were bulb-bearing. The folds were not separable into primary and secondary lamellæ as in the Leporidæ, but were occasionally cleft for half their depth into two quite equal portions, the interspace thus left being nearly free from epithelium. The furrows separating the folds varied greatly in width. Some of them were narrow above, but very much dilated below, while others were narrow and of uniform breadth throughout. The depth was fairly uniform, averaging 0.65 mm. Serous glands were exceedingly abundant, occurring within the papillæ as well as around them. The ducts were plentiful, many of them being of small diameter but of unusual length. They open at the bottom of the furrows and also at the sides of the folds at different levels. A small bundle of striated muscle-fibres terminated within the base of each fold. The bulbs are disposed on the lateral area of the folds in seven to nine tiers. They are likewise irregularly scattered in the epithelium bordering their mid-furrow. In horizontal section they are very closely packed. They measure 0.054 mm. in length and 0.030 mm. in breadth, the gustatory pore having a diameter of 0.0028 mm.

The fungiform papillæ bear bulbs. Some of them possess

unusually long necks, and apparently reach the free surface. The mucous glands of Nuhn are present in the tip of the tongue, their ducts opening on its inferior surface. The epithelium of the larynx was searched for bulbs, but none were detected.

In many ways the gustatory structures of *Castor fiber* approach closely those of *Arctomys monax*.

THE TONGUE OF *Cynomys ludovicianus*.

The material consisted of two specimens. One of the tongues had been kept in dilute spirit, and was gradually hardened in ordinary spirit and absolute alcohol. The other tongue, a fresh specimen, was prepared for microscopical examination by the method employed for *Lepus* and *Castor*.

General Description. — The organ is 41 mm. in length, 11 mm. in breadth, and 8 mm. in thickness. The free portion measures 16 mm. in length. The papillate surface is wrinkled anteriorly, and soft and velvety to the touch. The tip is obtuse, and slightly cleft as in *Arctomys*, *Fiber*, and *Hesperomys*. The fungiform papillæ are small and inconspicuous, but are quite evenly distributed over the anterior third and tip of the tongue. The filiform papillæ resemble those of *Fiber*. The under surface of the organ is impressed by a deep central groove. The circumvallate papillæ are three in number, and are situated well back on the dorsum. They are arranged in an isosceles triangle, the apex of the triangle being directed backwards. The posterior papilla is 3.5 mm. from the base of the tongue. The lateral gustatory organs are situated somewhat further forward than the circumvallate papillæ. When viewed from above, they present a row of slit-like openings, below which are a number of mound-like elevations. Each elevation is pierced near its centre by a small aperture, which proved on closer examination to be the mouth of a mucous duct. Above the lateral taste organ is a fringe of filiform papillæ, the points of which are directed upwards, inwards, and backwards. The fringe is continued on to the lower part of the glosso-palatine arch.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ. — The lingual surface adjacent to the papillæ is slightly papillate. The papillæ are of good size

and quite movable. They measure 1 mm. in diameter and 0.55 mm. in height. Their free surface is flattened or slightly convex, and they are occasionally cleft at their centre. The trenches are narrow and uniform. Serous glands are not very plentiful. Their ducts appear to open into the trenches at all levels. The bulbs are disposed at the lower half of the lateral area in six or seven tiers. I counted sixty bulbs in a tier, but the circle was not quite complete. The bulbs are closely packed in the tiers, and are fairly uniform in size and shape. They measure 0.052 mm. in length and 0.030 mm. in breadth.

The Lateral Gustatory Organs.—These organs are about 2.40 mm. in length, and consist of five or six irregular folds of the mucosa. The folds bear many secondary papillæ, and the epithelium covering them is very much thicker on the upper surface than at the sides. The furrows vary in depth, but average about 0.30 mm. The serous glands and ducts offer nothing unusual either in their position or structure. The bulbs occupy a large portion of the lateral area of the folds, there being from three to five tiers of them. They measure 0.051 mm. in length and 0.032 mm. in breadth.

Most of the fungiform papillæ contain bulbs. I found one remarkably fine specimen in this region. It measured 0.056 mm. in length and 0.036 mm. in breadth, and was almost wholly epithelial in position.

Bulbs occur in the epithelium of the epiglottis and elsewhere in the larynx. Those of the anterior surface of the epiglottis are more or less spheroidal, and have a diameter of 0.042 mm. Those of the posterior surface vary in length from 0.033 to 0.036 mm. Bulbs are also present on the inner surface of the thyroid and arytenoid cartilages.

THE TONGUE OF *Tamias striatus*.

I received fresh specimens of this tongue. They were prepared for histological examination by the method employed for *Lepus*, *Castor*, etc.

General Description.—The tongue is 22 mm. long, 6 mm. broad, and 5 mm. in thickness. The length of the free portion is 9 mm. The papillate surface is marked anteriorly by a faint mesial raphé. The dorsum in front of the circumvallate area is covered with small recurved papillæ, not unlike those of *Fiber*.

The apex of the tongue is obtuse, and the under surface smooth and unmarked by ridge or groove. Papillæ of the fungiform type are thinly distributed over the dorsum. They appear to be of normal structure. The circumvallate papillæ, three in number, are situated on the same transverse line. They are very near together, and are 3.5 mm. distant from the base of the organ. The lateral gustatory organs are quite far forward. A line dividing the circumvallate papillæ at their centres, if continued on to the under surface of the tongue, would pass through the posterior limits of the lateral taste organs.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ.—The papillæ are 0.44 mm. in diameter and 0.35 mm. in height. They are flattened on top, and frequently cleft at the centre. Their sides are quite symmetrical, and at the lower part bend slightly inwards. The trench encircling each papilla is deep and narrow. Serous glands are not very plentiful in the underlying stroma. The ducts open into the trenches at their deeper part. The bulbs are disposed in five or six tiers, filling completely the overhanging wall of the papilla. There are about fifty closely packed bulbs in a tier. They are fairly uniform in size, measuring 0.051 mm. in length and 0.029 mm. in breadth.

The Lateral Gustatory Organs.—The organs measure 2.10 mm. in length, and consist of seven or eight bulb-bearing folds. The folds are quite simple in construction, but vary somewhat in size. They are separated by deep and narrow furrows, the average depth being about 0.40 mm. Serous glands are not abundant. Their ducts open at the usual places. The bulbs are arranged in six to eight tiers, filling the lower half or lower two-thirds of the folds. They measure 0.048 mm. in length and 0.024 mm. in breadth.

All of the fungiform papillæ examined contained bulbs. One vertical section of a papilla showed two of equal size, measuring 0.039 mm. in length and 0.030 mm. in breadth. They were placed obliquely near the summit, with their bases resting in a depression of the mucosa, and their apices directed upwards and slightly outwards.

Bulbs were present in the epiglottis and occurred elsewhere

in the larynx. They measure in this region about 0.039 mm. in length and 0.024 mm. in breadth.

THE TONGUE OF *Sorex cooperi*?

The material consisted of spirit specimens.

General Description. — The organ is 11 mm. long, and is free from the frænum for 5 mm. The posterior portion is somewhat raised above the level of the anterior, and the dorsum is transversely ridged to fit the palatal grooves. The upper surface and sides are beset with small, densely packed, filiform papillæ. The papillæ are 0.15 mm. in height, and bear on their upper part sharp-pointed, recurved, cornified spines. The two circumvallate papillæ lie in the same plane, and are 0.5 mm. apart and 3 mm. from the base of the organ. As seen from above they are elliptical in form, and placed obliquely to the long axis of the tongue, with their anterior extremity directed outwards. The fungiform papillæ are very small and scattered. Nothing could be detected of the lateral gustatory organs. They are doubtless present, though probably of a simple type.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ. — The papillæ have a transverse diameter of 0.14 mm., and are 0.15 mm. in height. They are flattened or concave on top, and project but slightly from the opening of the trench. The sides converge somewhat sharply at their lower part, giving the papillæ a constricted base. The trenches are narrow and quite uniform in breadth. Serous glands are quite widely distributed through the mucosa and submucosa, but are not very plentiful directly beneath the papillæ. Their ducts discharge at the usual places. The bulbs resemble those of the Rodentia. They are arranged in three or four tiers at the lower half of the lateral area, and measure 0.033 mm. in length and 0.020 mm. in breadth.

The fungiform papillæ bear bulbs at their upper part. The epiglottis was not examined.

THE TONGUE OF *Blarina brevicauda*.

This tongue, a spirit specimen, was not in a suitable condition for minute study, and I am only able to give a very brief general account.

General Description. — The organ measures 15 mm. in length, 4.5 mm. in breadth, and 3 mm. in thickness. It is free from the frænum for 5.5 mm. Papillæ of the fungiform type are not numerous. The two circumvallate papillæ are on the same transverse line, and are about 3 mm. from the base of the tongue. They are clearly defined, and are 1.2 mm. from each other and about the same distance from the lateral limits of the organ. Their summits are circular, and measure 0.25 mm. in diameter. Serous glands are not abundant. The epiglottis was examined microscopically, but no bulbs were detected.

THE TONGUE OF *Scalops argentatus*.

The material consisted of spirit specimens.

General Description. — The organ is 25 mm. long, 7.5 mm. wide, and 6 mm. in thickness. The free portion is 13 mm. in length. The papillate surface is somewhat convex, and marked by seven or eight transverse ridges corresponding to the palatal grooves. The tip is rounded. The dorsum is covered with thickly set, simple and compound filiform papillæ of the ordinary type. Fungiform papillæ are not numerous, but are distributed quite uniformly over the anterior two-thirds of the dorsum. At about 5 mm. from the base of the organ are two elliptical-shaped circumvallate papillæ. They are set obliquely to the long axis of the tongue, with their anterior extremity directed outwards. The lateral gustatory structures were not easily detectable. They are situated as usual at the sides of the base of the organ, a little anterior to the circumvallate papillæ, and consist of a few simple, more or less irregular folds of the mucous membrane.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ. — The papillæ are lobate. They measure 0.90 mm. in diameter and 0.40 mm. in height. Both serous and mucous glands appear to be plentiful around the papillæ. No regular arrangement of the bulbs could be determined from my sections. They measure 0.048 mm. in length and 0.027 mm. in breadth.

The fungiform papillæ are well supplied with bulbs. Some of them are very small, being only about 0.021 mm. in length. They measure on the average, however, 0.035 mm. in length and 0.024 mm. in breadth.

The epiglottis and anterior surface of the larynx contain bulb-like bodies. They are almost wholly epithelial in position, and measure 0.036 mm. in length and 0.024 mm. in breadth.

THE TONGUE OF *Pteropus pselaphon*.

I examined two tongues of this species, which is peculiar, I think, to the Bonin Islands. They had been kept in spirit and were well preserved.

General Description. — The organ measures 48 mm. in length, 14 mm. in breadth, and 10.5 mm. in thickness, and is free for 25 mm. from the frænum. The extreme posterior dorsal region is more or less furrowed, and at the sides of the base are numerous large filiform papillæ, curving upwards, inwards, and backwards. The anterior dorsal surface is covered with closely packed, recurved filiform papillæ which, when stroked in the opposite direction, convey the feeling of a fine-toothed rasp. The tip of the organ is nearly pointed. The under surface is smooth, and marked by a median groove extending from the frænum half-way to the tip. The fungiform papillæ are of good size, but not very abundant. They are thinly scattered over the dorsum, and are also collected into a line at the sides, just above the junction of the papillate and non-papillate surfaces. The circumvallate papillæ, three in number, form a nearly equilateral triangle. They are very close to the base of the tongue, the papilla forming the apex of the triangle being but 3 mm. distant from it. The anterior papillæ are somewhat larger than the posterior papilla, and are about 1 mm. apart.

The Mechanical Papillæ. — The compound filiform papillæ of *Pteropus* approach quite closely in their structure the corresponding papillæ of the Marsupialia, of which they appear to be a modified form. They are large and prominent along the middle of the dorsum, but anteriorly and laterally they are smaller and more delicate, the transition from one form to the other being somewhat abrupt. Anteriorly there are from eight to ten papillæ to the square millimetre of surface. Posteriorly they are somewhat less thickly set. The larger ones measure at their base 0.40 mm. in diameter. A little above the base the diameter is very much less. Those near the tip have a transverse diameter of 0.20 mm., and are about 0.45 mm. in height. Each papilla is seated upon a single main papillary upgrowth of

the mucosa. Covering the papilla is a dense mass of stratified epithelium. No separation of the epithelium into distinct layers was possible in my sections. The secondary papillæ or processes are eight to twelve in number. At the lower part of the papilla they are grouped near the centre of the main up-growth. At higher levels they form an incomplete ring round the edge, leaving a horseshoe-shaped space within. The free ends of the secondary papillæ are very short, and completely cornified. They terminate in sharply pointed spines, the apices of which are directed inwards and slightly backwards. The secondary papillæ of *Pteropus* separate from the main papillary body at a very much higher level than in the Marsupialia, and their free portion is consequently much shorter. The terminal hooks are also relatively stouter in the former than in the latter, and more completely cornified. The papillæ are largest and fullest developed along the central portion of the dorsum. Towards the lateral border and tip they lose the ring of secondary processes, the latter being replaced by one or more short spines.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ. — These papillæ measure 1.25 mm. transversely, and are 0.60 mm. in height. Their summits are convex and slightly verrucose, and overtop the adjacent lingual area. The trenches are rather narrow, but not deep. Serous glands are very abundant within the papillæ and beneath and around them. The ducts are numerous and usually open at the bottom of the trenches. Those of the intrapapillary glands traverse the epithelium of the lateral wall, and open at a higher level. The bulbs of this taste area occupy the overhanging wall of the papilla, but are not entirely confined to it. They occur in the epithelium of the outer wall of the trench, and isolated ones are present on the lateral slopes of the upper surface of the papilla. Those of the overhanging wall are quite closely packed, and are disposed in a zone of eight to ten tiers. On the outer wall their arrangement is less regular. In a well-filled tier of the papilla there appear to be at least eighty bulbs. In a transverse section through the lower part of the outer wall I counted one hundred and seventy-five bulbs; the section, however, included parts of several tiers. The bulbs are long

and slender, and measure 0.060 mm. in length and 0.029 mm. in breadth.

The Lateral Gustatory Organs. — There is a somewhat striking resemblance noticeable between the gustatory folds of some of the Chiroptera and a circumvallate papilla of the usual type. In *Pteropus* this resemblance is less marked than in the genus *Vespertilio*, the gustatory structures of which have already been described in this *Journal* by the present writer. The organs consist of three or four very irregular folds of the mucosa. The intervening furrows are fairly uniform in width, but vary in depth, the average depth being about 0.60 mm. Serous glands are not very plentiful in this region. Their ducts open at the usual places. The bulbs are restricted to the lateral walls of the folds, and not infrequently nearly fill them. I counted upwards of twenty tiers, but the mean number is somewhat less. The bulbs are of the same shape and size as in the circumvallate papillæ. The marginal fringe of filiform papillæ, peculiar to this region in many mammals, has already been alluded to.

The fungiform papillæ are well supplied with bulbs, and they are also exceedingly numerous in the larynx, especially on the inner surface of the cricoid and arytenoid cartilages.

THE TONGUE OF *Nyctinomus nasutus*.

The specimens had been kept in alcohol, and were in a fairly good condition.

General Description. — The shape of the organ suggests a division into an anterior and a raised posterior part. The posterior division is 6 mm. in length, the total length of the organ being 11 mm. The anterior division is 4.2 mm. in breadth, 3 mm. in thickness, and is free from the floor of the mouth for 3.5 mm. The fungiform papillæ are quite large on the raised portion of the organ directly in front of the gustatory area. They are also arranged in a line (for a part of the distance in two rows) on each side of the tongue, at the junction of the upper and lower surfaces. The circumvallate papillæ, two in number, are situated one on either side of the median line, 1 mm. distant from the base. No search was made for the lateral gustatory organs for want of sufficient material.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ.—The papillæ are 0.30 mm. in diameter and 0.25 mm. in height, and project somewhat from the opening of the trench. They are flattened on top with symmetrically sloping sides curving downwards and inwards, the bases of the papillæ in consequence being somewhat constricted. The trenches are uniform in breadth and fairly deep. Serous glands are only sparingly present. There are five or six tiers of bulbs, the uppermost tier sometimes being on a level with the opening of the trench. In horizontal section I counted fifty bulbs. They measure 0.050 mm. in length and 0.028 mm. in breadth.

All of the fungiform papillæ examined bore bulbs, but they were smaller than those of the circumvallate papillæ. Bulbs were also plentiful in the epithelium of the epiglottis and elsewhere in the larynx.

THE TONGUE OF *Antilocapra americana*.

The material consisted of a single fresh specimen. It was hardened by the method adopted with *Lepus*, etc.

General Description.—The organ measures 180 mm. in length, and possesses the general characters of the ruminant tongue. The fungiform papillæ resemble black or dark-colored beads, and are quite numerous, especially about the tip, including its inferior portion. They are also arranged lineally on each side of the dorsum from the tip to the gustatory area, where they are not easily distinguished from those of the circumvallate type. The circumvallate papillæ are grouped in two main portions, on each side of the median line, at the posterior part of the dorsum. There appear to be about twenty-six papillæ on a side, those most posteriorly placed being 34 mm. from the base of the tongue.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ.—The papillæ are flattened or slightly rounded on top, and their sides are vertical or nearly so. The epithelium investing the lateral area is less thick than that of the upper surface, the latter in turn being much thinner than that of the adjacent lingual area. The papillæ measure

1.20 mm. transversely, and are 1.10 mm. in height. At their upper part they bear many small secondary papillæ. The trenches are narrow and of uniform width. Serous glands are plentiful, and their ducts usually open at the bottom of the trenches. (A few of the papillæ show a marked reversion to an earlier type. They consist merely of a simple semicircular ridge, partially roofed over above, and bearing bulbs over its circumference.) The bulbs are disposed in several tiers. In some papillæ the tiers are quite closely set, while in others there is considerable space between them. When closely set they are usually restricted to the middle portion of the lateral wall, the space above and below being destitute of them. The mean number of tiers is about twelve, and there are upwards of eighty bulbs in a tier. They measure 0.069 mm. in length and 0.032 mm. in breadth.

No lateral gustatory organs were detected. Thus far in the Ruminantia they have only been found, I think, in *Tragulus javanicus*, *Camelopardalis giraffa*, and *Antilope mergens*.

The fungiform papillæ are of the usual type and bear bulbs. The latter, however, appear to have relatively decreased in number, this perhaps being due to the unusual extent of bulb-bearing surface offered in the gustatory area proper of the tongue.

A few bulbs are present on the anterior surface of the epiglottis. Mucous glands are also abundant in this region.

THE TONGUE OF *Lutra canadensis*.

I received several specimens of this tongue, all but one of which were fresh. The fresh specimens were prepared by the method employed for the preceding tongue. The spirit specimen was from a very young animal, and the hardening was completed in ordinary alcohol.

General Description.—The organ is 71 mm. long, 23 mm. wide, and 5 mm. in thickness. Anteriorly it is much tied down, the free portion being only 15 mm. in length. The upper surface is covered with filiform papillæ, the apices of which are directed backwards. When stroked in the opposite direction, the papillæ convey the feeling of a very fine rasp. A wide but shallow median furrow leads from the area of the circumvallate papillæ to the tip. The circumvallate papillæ, seven or eight

in number, are arranged in the form of an inverted V. Fungiform papillæ are grouped quite thickly in the space bounded by the circumvallate papillæ, and are also scattered elsewhere on the dorsum. The tip of the organ is obtuse, and the under surface smooth and marked by a narrow groove extending from the frænum to the apex.

In the young *Lutra* the dorsal surface, anterior to the circumvallate area, was thickly studded with fungiform papillæ. They were very evenly distributed, but were most thickly placed along the median furrow, which in this tongue was well marked. Far back on the dorsum were six or seven circumvallate papillæ.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ.—The papillæ are 0.55 mm. in diameter and 0.75 mm. in height. The trenches encircling them are narrow and deep. Glands of the serous type are not very abundant. The ducts open into the trenches at different levels. The papillæ of the young *Lutra* were in better condition for studying the arrangement of the bulbs than those of the adult specimens. In the former the bulbs are disposed on the lateral area in six to eight tiers. They are also present in the epithelium of the outer wall, and even occur on the free upper surface of the papillæ. From horizontal sections there appear to be about fifty bulbs in a tier. They measure 0.053 mm. in length and 0.030 mm. in breadth.

The lateral gustatory organs appear to be rudimentary in structure. They consist merely of a few simple folds of the mucous membrane. The folds were destitute of bulbs, and no glands of the serous type were detected in this region.

The fungiform papillæ contained bulbs as usual. One from a papilla of the mid-dorsal region measured 0.045 mm. in length and 0.027 mm. in breadth. The base of the bulb rested against the mucosa, and the apex penetrated the superficial layers of the epithelium. No bulbs were detected in the epiglottis.

THE TONGUE OF *Canis lupus*.

This specimen and the following one were hardened in Müller's fluid and alcohol, according to the method already described.

General Description. — The organ is 156 mm. in length, 45 mm. in breadth, and 20 mm. in thickness. Anteriorly it is free from the floor of the mouth for 73 mm., or nearly half its length. The fore part of the papillate surface is impressed by a well-marked mesial groove. Posteriorly the groove is continued as an indistinct raphé to the base of the organ. The fungiform papillæ are quite evenly distributed over the dorsum and sides from the circumvallate area to the tip. They resemble minute white beads, being largest posteriorly and gradually decreasing in size as they approach the anterior limits of the organ. The extreme posterior region and sides are covered as usual with coarse, fleshy, recurved papillary elevations. The under surface, except at the margins, which are somewhat wrinkled, is quite smooth. The circumvallate papillæ are two in number. They are on the same transverse line, 13 mm. apart, and 26 mm. from the base of the tongue. At a short distance from the base, near the line of union of the upper and lower surfaces, the lateral gustatory organs are easily distinguishable. A line dividing the circumvallate papillæ at their centres, if continued on to the under surface of the tongue, would pass through the posterior end of the lateral taste organs.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ. — The papillæ are laterally involuted, and measure 1.25 mm. in diameter and 0.90 mm. in height. Their summits are more or less verrucose, and project somewhat from the trenches. The sides of the papillæ are quite symmetrical, and the trench encircling each is uniform in width. Serous glands are sparingly present, and their ducts open usually at the bottom of the trench. The bulbs are disposed in sixteen closely packed tiers, filling the inferior half of the papillary wall. A few bulbs are scattered at irregular intervals on the outer wall of the trench, and, from some indications, it is not improbable that isolated ones may also occur on the upper surface of the papilla. Judging from horizontal sections, there appear to be at least ninety bulbs in a tier. They measure 0.060 mm. in length and 0.031 mm. in breadth.

The Lateral Gustatory Organs. — These organs are 7 mm. in length, and consist of nine or ten irregular folds. The furrows vary in width and depth, the average depth being about 0.55 mm.

Serous glands are fairly abundant, and their ducts discharge as usual. The bulbs are disposed at the sides of the folds in some fourteen tiers. They vary but slightly from those of the circumvallate gustatory area, and measure 0.060 mm. in length and 0.029 mm. in breadth.

The bulbs of the fungiform papillæ are rather small and not very numerous. They are placed as usual. The larynx was not examined.

THE TONGUE OF *Canis latrans*.

General Description. — The organ measures 125 mm. in length, 30 mm. in breadth, and 20 mm. in thickness. It is free from the frænum linguæ for 38 mm. The upper surface is marked for nearly its entire length by a mesial groove or raphé. At the extreme basal region are the usual fleshy elevations, and the under surface presents a more or less wrinkled appearance. The fungiform papillæ are largest and most closely placed just anterior to the circumvallate area, and are smallest along the median groove and about the tip. The circumvallate papillæ, seven in number, are arranged in two rows, slightly converging posteriorly, one on each side of the median line. The lateral gustatory organs are placed nearly as in *Canis lupus*, their posterior limits being on the same transverse line as the anterior pair of circumvallate papillæ.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ. — The papillæ, like those of *Canis lupus*, are laterally involuted. They vary greatly in size, but average about 0.95 mm. in diameter and 0.80 mm. in height. The adjacent lingual surface is papillate, and the papillæ themselves are more or less verrucose and occasionally cleft. Serous glands are not very plentiful. Their ducts discharge at various levels. The bulbs of the papillæ are limited to the lower third of the lateral area, where they are disposed in ten to twelve closely packed tiers. They are also irregularly scattered in the outer wall of the trench. There are about sixty-five bulbs in a tier of the papilla. They measure 0.058 mm. in length and 0.033 mm. in breadth.

The Lateral Gustatory Organs. — These organs measure 5 mm. in length. Each consists of seven or eight folds varying slightly

in size, but having the same general appearance. The furrows have an average depth of 0.45 mm. Serous glands are fairly plentiful, and their ducts open at all levels. The bulbs are disposed at the sides of the folds in ten or more tiers, and also occur sparingly on their upper surface. They are a trifle smaller in this area than in the circumvallate papillæ.

The fungiform papillæ are well supplied with bulbs, sometimes as many as eight being visible in a single section of a papilla of this type. The epiglottis was not examined.

THE TONGUE OF *Canis familiaris*.

General Description.—The organ possesses a considerable extent of free portion. The under surface is smooth, and impressed by a median groove extending from the frænum half-way to the tip. From this point it is continued as a slight ridge to the apex. The tip, which is obtuse, is very slightly bifid. The upper surface is marked anteriorly by a mesial raphé. Papillæ of the fungiform type are quite evenly distributed over the dorsum, and the usual fleshy elevations project from the basal surface of the tongue. There are four to seven circumvallate papillæ, arranged very much as in *Canis latrans*. The lateral gustatory organs are clearly defined.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ.—The papillæ vary in size, but their transverse diameter is almost always greater than their height. Not infrequently they are cleft vertically. The trenches are narrow and uniform in width. Serous glands are not very abundant. The ducts open into the trenches at their base and deeper part. The bulbs are disposed on the lateral area in ten to fourteen closely packed tiers. They also occur irregularly on the outer wall of the trench, near its upper part. They are likewise present in the epithelium lining the vertical cleft of the papillæ, and, according to Ditlevsen, may sometimes be found on their free upper surface. They are densely packed in the tiers, the tiers of the larger papillæ containing upwards of one hundred and thirty bulbs. In the smaller papillæ the number in a tier is about eighty. The bulbs vary in length from 0.060 to 0.071 mm., and in breadth from 0.025 to 0.035 mm.

The Lateral Gustatory Organs.—These organs vary from

3.70 to 10 mm. in length. They consist of seven or eight folds, most of which bear bulbs on one or both lateral areas. In some folds the main papillary body is cleft into a few large secondary papillæ, the depressions between which are filled up to one level with epithelium. The furrows are narrow, and average 0.50 mm. in depth. Serous glands are plentiful, the ducts opening at the bottom of the furrows. The bulbs occasionally fill the lateral area of the folds completely, and scattered bulbs are not infrequent on their exposed surface. Usually there are from ten to fourteen tiers on each lateral wall. I counted forty-six bulbs in a tier, but the latter was not completely filled, nor did it represent the entire width of the organ. The bulbs of this region vary in size, and are a little smaller than in the circumvallate papillæ. They measure 0.056 to 0.067 mm. in length and 0.026 to 0.035 mm. in breadth.

Some of the bulbs of the fungiform papillæ penetrate, and even perforate, the outer layers of the epithelium. I found one very beautiful flask-shaped specimen in this region. The basal end rested against the mucosa, while a very delicate, but clearly defined neck led to the exterior. The bulb measured 0.057 mm. in length and 0.028 mm. in breadth.

Both Davis and Schofield have found bulbs at different parts of the posterior surface of the epiglottis of *Canis familiaris*. They have also been detected by Davis, and later by Simanowsky, on the true vocal cords. The former investigator has likewise observed them on the inner side of the arytenoid cartilages and on the aryepiglottic folds.

THE TONGUE OF *Zalophus californianus*.

The specimen came from a young individual, and had been kept for some time in spirit.

General Description. — The organ is 58 mm. long, the length of the free portion being 14 mm. The tip is bifurcate. The under surface is finely wrinkled, and impressed by a deep median groove leading to the frænum. The anterior dorsal surface is marked by a slight mesial raphé. Fungiform papillæ are sparingly scattered over the anterior dorsal region, and the anterior margin and tip are fringed with filiform papillæ. Near the base of the organ are four or five papillæ of the circumvallate type. They are arranged in two unequal lines converging backwards.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ.—The papillæ are 0.90 mm. in diameter and 0.45 mm. in height. The trenches are not deep, and have a uniform breadth. Serous glands are very abundant, and occur within the papillæ themselves. The ducts open at or near the bottom of the trenches. They are remarkably straight and have a nearly uniform diameter. Some of them are quite long, occasionally exceeding two millimetres in length. The ducts of the intrapapillary glands pass through the epithelium of the lateral wall and open about opposite the middle of the trench. This being a young individual, the bulbs are present on the upper surface as well as upon the sides of the papillæ. In one section I counted twelve on the upper area. The arrangement of the tiers is very irregular. Isolated bulbs occur on the outer wall of the trench, especially at its upper part. The bulbs measure 0.054 mm. in length and 0.033 mm. in breadth.

The lateral gustatory organs were represented by five or six fairly symmetrical folds at the sides of the base of the tongue. There were, however, no indications of bulbs, and serous glands were likewise wanting in this region.

The fungiform papillæ were very well supplied with bulbs. The epiglottis was not examined.

THE TONGUE OF *Phoca vitulina*.

This tongue, of which I received a fresh specimen, was hardened in Müller's fluid and alcohol.

General Description.—The tongue is 95 mm. in length, its greatest transverse diameter is 51 mm., and it measures 30 mm. in thickness. The organ possesses but little mobility, the free portion being but 21 mm. in length, and there being but a small extent of free margin. The tip is bifurcate, and fringed with filiform papillæ. There is a slight groove, 5 mm. long, on the upper surface, and a narrower and much shallower one, 8 mm. long, on the under surface. They both terminate at the tip.

The anterior and middle portions of the dorsum are covered with large, closely set, cornified papillæ, the apices of which are directed inwards and backwards. The mucous membrane of the remaining portion of the dorsal surface is thrown into numer-

ous, wavy, sub-parallel folds. Those of the lateral borders have a more or less longitudinal direction, and are continued from each side of the tongue on to its under surface, meeting in front of the frænum. I found some difficulty in identifying fungi-form papillæ. There appear, however, to be a few of this type scattered here and there between the folds. The circumvallate papillæ are ten to twelve in number. They are in the form of an inverted V, the apex of the V being about 21 mm. from the base of the organ. No lateral gustatory structures were detected.

The folds of the dorsum measure on the average 0.60 mm. in height and 0.70 mm. in breadth. They are flattened or slightly convex on top, and are covered with a fairly uniform layer of epithelium. The epithelium is a trifle thicker on the upper surface than at the sides and, in places, appears to be somewhat cornified. The mechanical papillæ covering the anterior portion of the dorsum are wedge- or cone-shaped, and measure 0.50 mm. in height and 0.40 mm. in breadth. They bear at their upper part from one to three recurved spinules. The epithelium sheathing the papillæ is partly, and that composing the spinules wholly, cornified. Underlying the papillæ, and only separated from them by a thin layer of mucosa, is a large amount of fat in the form of lobules and groups of fat-cells.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ. — The papillæ vary greatly in size. One of the larger ones measured 2.30 mm. transversely, and was 1 mm. in height. At their upper part they break up into many secondary papillæ. In some papillæ the upper portion is cleft and furrowed. The trenches vary in width and depth. Nerve-fibres enter the papillæ at their base, and, towards their upper part, divide and form a rather coarse plexus. Serous glands are not very abundant. Their ducts, which are often very large, open at the bottom of the trenches. There is a great abundance of fat in the mucosa and submucosa, and groups of fat-cells also occur within the papillæ. The bulbs are far from numerous. They differ in size and shape, and are very irregular in their distribution. One of them measured 0.060 mm. in length and 0.036 mm. in breadth.

THE TONGUE OF *Hapale jacchus*.

This specimen and the two following had been preserved in spirit. They were none of them fully adult.

General Description. — The anterior dorsal surface of the organ is impressed on each side of the median line by four or five transverse furrows. The fungiform papillæ are quite large, though not very numerous. The under surface presents a wide and deep wedge-shaped groove extending from the frænum to the tip. The tip is obtuse. In *Quadrumana* the circumvallate papillæ are usually three in number. In this specimen, unfortunately, the tongue had been divided just behind the anterior pair, so that I had no opportunity of examining either the posterior papilla or the lateral gustatory organs. The two anterior papillæ are 3 mm. apart, and 1.5 mm. from the lateral limits of the organ.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ. — The papillæ are 0.33 mm. in diameter and 0.28 mm. in height. They are flattened on top, and their sides incline inwards at the base. Serous glands are not plentiful. Their ducts open as usual into the trenches at their deeper part. The bulbs are irregularly scattered over the upper surface and sides of the papillæ. From horizontal sections there appear to be about thirty-five in a tier. They are quite small, measuring only 0.039 mm. in length and 0.025 mm. in breadth.

The bulbs of the fungiform papillæ present the usual appearance. The epiglottis was not examined.

THE TONGUE OF *Macacus cynomolgus*.

General Description. — The organ measures 50 mm. in length, 16 mm. in breadth, and is free from the frænum for 15 mm. Fungiform papillæ of normal structure are thickly distributed over the dorsum, and also extend on to the under surface. Those about the tips are quite closely set. The circumvallate papillæ, of which there are two pairs, — an anterior and a posterior, — are situated well back on the dorsum. The posterior pair are 1 mm. apart, and 11 mm. from the base of the organ. The anterior pair are 6 mm. from the posterior, and are 10 mm. apart. The lateral gustatory organs are placed about as usual.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ. — The papillæ are of nearly equal size. Their summits are more or less flattened, and their sides symmetrical and nearly vertical. The trenches are narrow and deep. Serous glands are abundant, and their ducts open at or near the bottom of the trenches. The bulbs are numerous, and are disposed in a varying number of tiers. In some sections there appear to be not less than twenty tiers, but the mean is probably ten. The number of bulbs in a well-filled tier is about a hundred. They measure 0.058 mm. in length and 0.032 mm. in breadth.

The Lateral Gustatory Organs. — The lateral taste organs are 6 mm. in length, and consist of seven or eight bulb-bearing folds. The furrows are quite uniform in breadth, and average 0.70 mm. in depth. Serous glands and ducts are quite plentiful. The bulbs are disposed at the sides of the folds in nine or ten tiers, and measure 0.057 mm. in length and 0.031 mm. in breadth.

The fungiform papillæ contain many bulbs, those about the tip being particularly well supplied. In a section of a papilla of this region I have seen as many as twelve. No true bulbs were detected in the epiglottis.

THE TONGUE OF *Macacus rhesus*.

General Description. — The organ is 41 mm. in length, 15 mm. in breadth, and is free from the frænum for 13 mm. The tip is rounded, and covered with closely set fungiform papillæ. Papillæ of this type are also thickly distributed over the dorsum, and upon the sides and under surface to the lateral line of union of the papillate with the non-papillate surface. The circumvallate papillæ, three in number, are arranged in an isosceles triangle, the apex of the triangle being directed backwards. The posterior papilla is 11 mm. from the base of the organ. The papillæ viewed from above present a smooth, slightly oval surface. The lateral gustatory organs are placed more obliquely than in *Macacus cynomolgus*, their posterior end being nearly on a level with the upper surface of the tongue.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ. — The summits of the papillæ are smooth, but more convex than in *Macacus cynomolgus*. The trenches are narrow and of uniform breadth. Serous glands are not very abundant. The ducts open into the trenches at their deeper part. The taste bulbs are disposed at the sides of the papillæ in ten to twelve tiers. A few bulbs are also scattered over their upper surface. They are not very closely packed, there being only about fifty bulbs in a tier. They vary greatly in size and shape, but the mean length is 0.068 mm. and the mean breadth 0.036 mm.

The Lateral Gustatory Organs. — These organs consist of a few folds of the mucous membrane. The intervening furrows are narrow, and 0.60 mm. in depth. Serous glands are not abundant. The bulbs, of which there are ten or more tiers, measure 0.066 mm. in length and 0.036 mm. in breadth.

The fungiform papillæ are of normal structure, and are richly supplied with bulbs. One deeply placed bulb, lying directly in the long axis of a papilla, measured 0.062 mm. in length and 0.045 mm. in breadth, the canal leading from it to the exterior having a length of 0.016 mm. and diameter of 0.003 mm. Bulbs were also present on the anterior surface of the epiglottis.

CONCLUDING REMARKS.

Far-reaching generalizations, based upon the study of the gustatory organs of the widely separated forms (some of them scarcely typical of their kind) which have been considered in this paper, would be of very doubtful value. It is therefore my intention to say but little at this time, reserving further remarks for a future occasion.

In the Marsupialia the circumvallate papillæ are three in number. They are arranged in the form of an equilateral or isosceles triangle, the apex of which is directed backwards.¹ In some genera, as *Halmaturus*, *Macropus*, *Petrogale*, and *Dasyurus*, these papillæ might very justly be called gustatory ridges, from their general shape, extreme protection, and concealed position. In these respects, as well as in the arrangement of the bulbs, they resemble the ridges of *Ornithorhynchus ana-*

¹ In *Dendrolagus*, according to Owen (*Comp. Anat. and Phys. of Verts.*, vol. iii., 1869, p. 191), the apex of the triangle is turned forwards.

tinus. In other genera, as *Phalangista*, *Belideus*, *Acrobates*, *Bettongia*, *Phascolarctos*, and *Didelphys*, the posterior papilla follows more closely the type characteristic of higher animals, while the anterior pair are still in a transitional stage of development. In *Phascolomys*, *Perameles*, and some species of *Didelphys*, all three papillæ are closely allied to the type common to higher animals.

Although the lateral gustatory organs have not been detected in all marsupials in which a search for them has been made, they have been found in a sufficient number of species to render their existence in all scarcely open to doubt. In *Halmaturus*, according to Poulton, the organ consists of a row of gland ducts, in the walls of which scattered bulbs are developed. In *Macropus*, *Petrogale*, and *Phascolarctos* they are more advanced, the ducts opening at the bottom of depressions. In *Perameles* the organ consists of a single epithelial-lined furrow, in the walls of which are several tiers of bulbs. In *Phalangista*, *Belideus*, *Acrobates*, and *Didelphys* the organs are less simple in structure, and the ducts open at the bottom of the furrows as in the higher mammals.

Edentata have but two circumvallate papillæ. The posterior papilla has disappeared, to reappear in the Castoridæ, Sciuridæ, Pteropodidæ, and Quadrumana. They are on the same transverse line, and may be considered as representing the anterior papillæ of the Marsupialia. While some species show a very decided advance in all, or nearly all, the points which the papillæ of the two orders have in common, other species appear to follow closely the marsupial type. In *Dasypus peba* they resemble in the main those of higher animals. In *Chlamyphorus truncatus* they approach quite closely the marsupial type, the resemblance between them and the anterior papillæ of *Belideus* and *Phalangista* being very marked. The papillæ of *Dasypus villosus* appear to hold an intermediate position, both types being represented, although more or less modified. This species was the only one in which I detected lateral gustatory organs, although they are doubtless present in the others. The organs were wholly unlike any yet described except those of *Procyon lotor*.

Bulbs appear to be wanting in the larynx of both the Marsupialia and Edentata. Further research, however, may show their existence in this region, although it is not unlikely that their appearance here is more recent.

TABLE.

SPECIES.	Number of Circumvallate Papillæ.	Number of Bulbs in Circumvallate Papillæ.	Mean Dimensions of Bulbs.		Lateral Gustatory Organs.	Number of Bulbs in Lateral Gustatory Organs.	Mean Dimensions of Bulbs.	
			Length.	Greatest Transverse Diameter.			Length.	Greatest Transverse Diameter.
<i>Didelphys virginiana</i>	3	2,900	mm. 0.054	mm. 0.034	Present.	...	mm. 0.046	mm. 0.030
<i>Bettongia cuniculus?</i>	3
<i>Phascolomys wombat</i>	3	3,500	0.065	0.030
<i>Phascolarctos cinereus</i>	3	4,200	Ditto.
<i>Dasybus peba</i>	2	2,400	0.054	0.030
<i>Dasybus villosus</i>	2	2,500	0.051	0.030	Ditto.	...	0.042	0.024
<i>Dasybus minutus</i>
<i>Chlamyphorus truncatus</i>	2
<i>Lepus campestris</i>	2	1,200	0.049	0.030	Ditto.	16,800	0.049	0.030
<i>Geomys bursarius</i>	1	...	0.036	0.024	Ditto.	...	0.036	0.021
<i>Hesperomys leucopus</i>	1	...	0.048	0.024	Ditto.	...	0.042	0.024
<i>Castor fiber</i>	3	...	0.055	0.031	Ditto.	...	0.054	0.030
<i>Cynomys ludovicianus</i>	3	1,100	0.052	0.030	Ditto.	...	0.051	0.032

<i>Tamias striatus</i>	3	750	0.051	0.029	Ditto.	...	0.048	0.024
<i>Sorex cooperi</i> ?	2	...	0.033	0.020
<i>Blarina brevicauda</i>	2
<i>Scalops argentatus</i>	2	...	0.048	0.027
<i>Pteropus pselaphon</i>	3	3,500	0.060	0.029	Ditto.	...	0.060	0.029
<i>Nyctinomus nasutus</i>	2	...	0.050	0.028
<i>Antilocapra americana</i>	52	48,000	0.069	0.032	Wanting.
<i>Lutra canadensis</i>	7-8	2,400	0.053	0.030	Rudimentary.
<i>Canis lupus</i>	2	2,900	0.060	0.031	Present.	...	0.060	0.029
<i>Canis latrans</i>	7	5,000	0.058	0.033	Ditto.	...	0.057	0.033
<i>Canis familiaris</i>	4-7	8,000	0.065	0.035	Ditto.	...	0.062	0.033
<i>Zalophus californianus</i>	4-5	...	0.054	0.033	Rudimentary.
<i>Phoca vitulina</i>	10-12	...	0.060	0.036	Wanting.
<i>Hapale jachus</i>	3	...	0.039	0.025
<i>Macacus cynomolgus</i>	4	4,000	0.058	0.032	Present.	...	0.057	0.031
<i>Macacus rhesus</i>	3	1,800	0.068	0.036	Ditto.	...	0.066	0.036

THE ORIGIN OF THE TEST-CELLS OF ASCIDIANS.

T. H. MORGAN, PH.D.

A WORD of explanation and apology seems necessary on adding another account to the long list of descriptions of the origin of the test-cells. While studying the embryology of *Clavellina* in the spring of 1888, I became interested in the origin of the test-cells, and the work was continued during the summer of the same year. A preliminary note was written in October, '88, and published in the Johns Hopkins University Circular, Vol. VIII., No. 72.

At the same time I obtained the paper of Van Beneden and Julin in the Archives de Biologie, Tome, VI., '87, in which I found conclusions almost exactly similar to those to which I had come. It seemed then unnecessary to publish a full account of the work, and the figures and descriptions were laid aside.

In the spring of '89, Dr. M. v. Davidoff published a new account of the origin of the test-cells (Mittheilungen aus der Zoologischen Station zu Neapel), in which he differs essentially from Van Beneden and Julin. Thus the whole question became once more unsettled by the conflicting accounts of Van Beneden and Davidoff, and seemed worth working over again.

During the summer of '89 I carefully prepared ovaries of several Ascidians by the methods described in detail by Davidoff, hoping in this way to meet him to some extent on his own grounds, and to test the value of the new methods of preparation. During the winter of '89-'90 this material was examined, and gave not the slightest evidence of such an origin of the test-cells as described by Davidoff. Here again I was irresistably led to the same conclusions as those reached by Van Beneden.

As the results obtained by the methods described by Davidoff differed in no essential points from those obtained by other methods, it seemed unnecessary to draw all the figures from such preparations. Several genera were examined, including

Cynthia ocellata, *Cynthia partita*, *Ascidia amorpha*, *Molgula manhattensis*, *Perophora viridis*, *Amaræcium stellatum*, and *Clavellina* sp.?

Finally, during the summer of '90, a new method of preparation was obtained, which confirmed, from another point of view, the previous results, and helped to make clear the exact origin of the test-cells.

Cynthia ocellata. — The ova are arranged around a central cavity, which is the body cavity according to Van Beneden. This cavity, which communicates by a duct with the atrial cavity, is lined by a germinal epithelium, within which the ova originate.

In sections through the ovary, nuclei are seen lying along the wall of the cavity of the ovary; and here and there one is seen to have enlarged, and the protoplasm about it to have increased in quantity. This nucleus and protoplasm form the commencement of a new egg. Such a condition is shown in Fig. 1, Pl. VIII. At the periphery of this young egg another nucleus is seen. This nucleus is one of those peripherally lying nuclei which go to form the follicular nuclei of the egg. In Fig. 2 we see an older stage: at *f* is a nucleus of the forming follicle with its protoplasm stretching over the surface of the egg. Other and similar nuclei lie around the egg in other sections of the series. The follicular protoplasm surrounding the egg is not a continuous mass, but is split up into cells corresponding in number to the follicular nuclei. The cell walls are so extremely thin that at this stage they cannot be seen in such sections.

Passing to a later stage, as shown in Fig. 3, the egg is seen while enlarging to have pushed inwards from the germinal membrane, which is still, however, attached to one side of the egg.

The peripheral zone of protoplasm of the follicle is much wider than in the last figure, and now is seen clearly to cover the whole surface of the egg. Four follicular nuclei are seen in this section. The nucleus of the ovum has also enlarged, and contains in the figure a single large nucleolus.

Fig. 4 is part of an egg of *Cynthia partita* (hardened in picro-acetic acid). The follicular zone is wider than in the last figure, and the follicular nuclei are a little larger. The egg

has passed into the general stroma of the ovary. This is the stage in development just before the formation of the test-cells.

In the next figure (5) the test-cells have begun to appear. At *tc* the follicular zone is seen pushing into the substance of the egg, and a careful examination shows faint indications of cell outlines at this place. In other words, one of the follicular cells has changed somewhat its position, and has come to lie a little interior to the cells of the follicular zone. The cell contains a nucleus which agrees in all details with those of the follicle. At *tc'* another such cell is seen; and at *tc''* a cell of the follicular zone is seen pushing into the interior of the egg.

These three cells, which take a more internal position, are the follicular cells, which are in process of conversion into test-cells. Two main sources of error may arise in interpreting the sections at this stage and are carefully to be avoided.

Inner nuclei may be seen if the section passes — not through the centre, but — near one end of the egg, where the convexity of the surface is so great relatively to the plane of the section, that two or more layers of the nuclei of the follicle may appear in the same section. A careful count was made in all cases of the sections and the central ones chosen for study. Again, an error may arise when the microtome knife does not cut the egg cleanly, but turns over part of the follicular zone.

The stage in development in which the test-cells appear is very constant, and I have never succeeded in finding any nuclei or cells inside of the follicular zone before such a stage of development is reached. In Fig. 6 we see a cross-section of an egg of *C. ocellata*, in which the migration of test-cells is completed. The follicular zone is seen to be divided up into distinct cells, which are sharply separated from the egg. At the periphery of the yolk, and touching the follicular zone, lie the test-cells. These do not stain so deeply as the protoplasm of the egg, and in all respects resemble the cells of the follicle.

After a stage represented by Fig. 6 has been reached, the number of test-cells does not seem to increase; although between Figs. 5 and 6 it is probable that the test-cells divide at the periphery of the yolk.

In Fig. 6 there is seen at the outer periphery of the follicle

a number of small nuclei lying in close contact with the follicle cells. These seem to belong to the stroma of the ovary, and to be added secondarily to the growing ovum.

In later stages the test-cells do not seem materially to change either in number, or size, or structure, but the follicular cells continue to increase in size and become much vacuolated.

By the methods of preparation ordinarily employed, the boundaries of the cells of the follicle do not appear in sections of young ova. In order to determine the shape and arrangement of the follicular cells, I experimented with other methods and found one which made clear the structure of the cells, and indirectly confirmed the conclusions reached by previous methods of preparations. The fresh ovaries were teased apart in very dilute osmic acid, washed in distilled water, and placed in a one per cent solution of silver nitrate, where they remained for half an hour; then put into a two per cent solution of acetic acid for the same length of time, and placed in the sunlight. They were then examined under the microscope, and the cell boundaries were distinctly seen. The eggs were then carried through the usual processes, imbedded in paraffin, and cut into thin sections.

In young ova, the follicular cells were found from surface views to have irregular outlines, and in general appearance to resemble peritoneal epithelial cells. A bit of the periphery of the egg is shown in Fig. 7, which corresponds approximately to an egg at an age represented in Fig. 3.

As the egg increases in size, the outer surface area of each follicular cell diminishes; but it will be remembered they are increasing in thickness during this period. At the time when the test-cells are forming, corresponding to Fig. 5, the follicular cells have reached a size shown in surface view by Fig. 8. In Figs. 9 and 10 are drawn sections of the periphery of eggs at this stage, showing the origin of the test-cells. Here it is seen that one of the follicular cells pushes itself inside of the follicular zone and becomes a test-cell. Comparing Fig. 8 with 9 and 10, the size of the test-cells is seen to exactly correspond to that of the follicular cells; so that we find at the time when the test-cells are formed that the follicular cells have reached exactly the same size as the test-cells, and this by itself would lend probability to the view that the inner cells are derived from the outer. Taken in connection with what we learn

from sections, it seems to me to leave not the slightest doubt as to the origin of the test-cells. The protoplasm of the egg of *Cynthia* stains deeply, and the color cannot easily be extracted. In this respect it is unfavorable material to determine the truth of Davidoff's position; but in the next form, *Clavellina*, the protoplasm (and yolk) stains scarcely at all, and wandering nuclei could be easily observed were there any such in the egg between the nucleus and the follicle.

Clavellina sp.?—The ascidian from which the material for work was obtained was collected at Green Turtle Cay, Bahamas, but I have not identified the species. The eggs are extremely large and very favorable for study, both on account of their size and for other peculiarities which will be seen in the following description. On account of the large amount of food yolk in this American *Clavellina*, no better material could be obtained to test the correctness of Davidoff's theory. The test-cells are not formed till the egg is quite large, and has a good deal of yolk; so that if the nucleus of the test-cells came from the nucleus of the ovum, these migrating pieces would have to travel a long distance from the centre to the periphery of the egg, and the chances of seeing such a migration would be very great.

The young nuclei of the ovum arise in the germinal epithelium of the ovary, and are at first like the ordinary nuclei of the membrane. The earliest stage is shown in Fig. 11. A somewhat older ovum is shown in Fig. 12, in which the protoplasm about the nucleus has increased, and there is a sharper distinction between the egg proper and the more peripherally lying follicular nuclei. The protoplasm of the follicular nuclei is seen to be thickening over the surface of the ovum, beginning at that part of the periphery of the egg which is in contact with the germinal membrane. In Fig. 13 the ovum has left the surface of the cavity of the ovary and passed into its substance. The peripheral zone of follicular protoplasm has spread over the whole surface of the ovum, and the follicular nuclei, which are now more numerous than in the last figure, lie within this protoplasm. At this stage in the history of the ovum of *Clavellina* the yolk commences to form, and appears as a zone around the nucleus. It is seen in Fig. 12 as an irregular mass, which in the figure is colored a lighter shade than the surrounding

protoplasm. There is no sharp line of demarcation in the section between yolk and surrounding protoplasm, but the latter sends processes into the former.

Passing to a stage represented by Fig. 14, we have essentially the same conditions as in the last figure. The ovum has increased very much in size, and the increase is largely due to the accumulation of yolk. Around the periphery of the ovum is a zone of protoplasm which does not contain yolk, and outside of this is the follicular protoplasm with its nuclei. As a rule, the nucleus of the ovum lies near to the centre of the egg, but in the section from which the figure was drawn it was near one end of the egg. It was also irregular in outline, but this is exceptional at this stage. In Figs. 15 and 16 we have the first indication of an origin of the test-cells. In Fig. 15 only a part of the egg is shown, but it will be easily seen that there has been a very great increase in size as compared with Fig. 14. At the periphery of the yolk lies a narrow zone of protoplasm, and the follicular protoplasm around the egg is wider than in the last figures. Here and there a nucleus lies nearer to the inner part of the zone, and it is generally accompanied by an ingrowth of protoplasm from the follicular zone. These ingrowths are the first trace of the formation of the test-cells. Where they project into the ovum, they are clearly seen to push through the peripheral zone of protoplasm of the egg.

The follicular zone stains differently from the protoplasm of the ovum, and one thus distinctly sees that the ingrowth of protoplasm with its contained nucleus belongs in each case to the follicular zone and represents migrating cells into the protoplasm of the egg. In Fig. 16 we see three stages in the ingrowth of follicular cells. The earliest is shown at tc , where an ordinary follicular nucleus has moved a little interior to the general row, and at the same place the follicular protoplasm has become thickened on the inner side. At tc' the follicular nucleus has passed still further within, and the surrounding protoplasm projects further into the egg, passing through the zone of protoplasm. At tc'' we see a somewhat exceptional condition, owing to the large amount of follicular protoplasm which has pushed into the ovum. It has passed the zone of protoplasm of the egg, and projects into the yolk substance. Within it and at its base lies a follicular nucleus. In later stages all of

these ingrowths become constricted off to form the test-cells. After separation from the follicular zone, the test-cells multiply at the periphery of the egg, and form each clusters of four or five cells closely connected.

In Fig. 17 is shown a section through the periphery of the egg, taken at a time when the test-cells were entirely within the follicular zone. Three such cells are seen in the figure, and it will be also noticed that at the same time the peripheral zone of protoplasm of the egg becomes extremely narrow, and at the time of its disappearance a membrane appears around the egg between the follicular cells and the yolk, and enclosed within this membrane are the test-cells. Most probably this peripheral protoplasm either secretes the membrane before its disappearance, or becomes converted into it.

In Fig. 18 is drawn a part of the periphery of an egg of *Clavellina* when the egg is nearly mature. The test-cells are seen lying in clusters of from one to a dozen at the periphery of the yolk and just within the egg membrane. Outside of the membrane the columnar follicular cells form a layer completely investing the egg, and beyond the follicular cells is an imperfect layer formed of scattered cells which seem to be derived from the inner substance of the ovary, although I have not carefully traced their origin.

The account I gave of the formation of the test-cells of *Clavellina* in my preliminary note is not quite clear nor entirely free from error. This is due to my not distinctly recognizing that the peripheral zone of protoplasm of the egg belonged entirely to the ovum; but I hope the preceding account will make clear the true position of the egg membrane.

It seems useless to give an extended criticism of the results and theory of Davidoff. Our views are diametrically opposed, and it seems to me irreconcilable.

My own results agree essentially with those of Van Beneden and Julin, and must stand or fall with theirs. Those who care for a fuller knowledge of Davidoff's position can refer to his paper (in the *Mittheilungen aus der Zoologischen Station zu Neapel*. Neunter Band. I. Heft. 1889). I will give here very briefly an outline of his views, so that I may contrast them with the preceding account.

In very young eggs (much younger than those in which I

have found the test-cells forming) Davidoff believes the nucleus to constrict off portions of itself which he calls buds (Knospen Nucleogemmæ, Kernknospen). These nucleogemmæ migrate to the periphery of the egg, and divide in many cases into smaller nuclei, although *no mitotic, nuclear figures were found*, and the more peripheral nuclei stained more deeply than the more central bodies. At a later stage of development (which corresponds to a stage in which I have found the test-cells constricted off from the follicular zone) Davidoff finds these peripheral nuclei — nucleogemmæ — *to multiply by mitotic division*. At the stage when these karyokinetic figures are found, the periphery of the egg is colored more intensely than elsewhere. Then the peripheral protoplasm of the egg divides itself into cells with a nucleogemma in each such cell.

Next follows a short account of the formation of the ova of *Fritillaria*. The young ovary contains a few cells which he calls oöblasts. They subsequently fuse, forming a syncytium. From the nuclei of this syncytium — the karyoblasts — there are budded off nucleogemmæ which, after cutting off some of the protoplasm of the syncytium, form the true ova. Comparing the Ascidian with the Appendicularian developments, Davidoff concludes, “Bevor wir indessen diesen Vergleich mit Erfolg durchführen können, müssen wir uns die Frage vorlegen, was denn das Ei der Ascidien selbst ist. Nach allem Gesagten kann dasselbe nicht mit einem Ei der Fritallaria homologisirt werden. Wir müssen viel mehr zuruckgreifen und sehen, ob wir die Ascidien-Eier mit einem der Ooblasten des Fritallaria — Ovarium vergleichen können. . . . Wir können also jetzt mit Recht den Schluss ziehen, *dass das Ei der Ascidien eigentlich kein solches in gewöhnlichen Sinne ist*. Es ist vielmehr ein Oöblast, welcher erst Eier producirt.” These eggs are the test-cells, or, as Davidoff concludes to call them, abortive eggs. By some such complicated explanation with its complicated terminology, he attempts to homologize the formation of the ova in the Appendicularian and Ascidian. Such a process of formation of the Ascidian egg seems so highly improbable in itself, and the method by which the oöblast divides into abortive eggs and egg proper is so out of the usual course of events, that it must take exceedingly strong evidence to support such a position. On the other hand, the increase in area of the follic-

ular cells by a process in which some of the cells are pushed interior to the others is easily understood. We see in the oögenesis of the squid a parallel case, where the process is carried to even a greater extent, the substance of the ovum being permeated by the trabeculæ of the follicular zone. In the squid there being many cells pushed in at several points, and in the Ascidian a single cell here and there over the surface.

The origin of the ova in vertebrates at the surface of the ovary, and their migration into its stroma, accompanied by follicular cells, themselves derived from the peripheral cells of the ovary, and their subsequent arrangement around the young egg into two or more layers, furnishes a most striking resemblance to the origin of ova, and follicular and test-cells, of the Ascidians.

In Davidoff's paper he says that Van Beneden, who examined his first series of slides, concluded that in *Distalpa* the test-cells, after originating from the follicular cells, migrated into the yolk. If this be true, then the difficulties of determination of the origin of the test-cells would be greatly increased, from his own point of view. And as in the origin of the test-cells it is not conceivable that in one Ascidian we have true ova, and in another oöblasts to replace them, when all the other processes are identical, no explanation of our work being done in different genera seems possible. After having gone over again and again sections of ovaries prepared by many methods, I have been as often forced to accept the account I have given in the first part of this paper, both because no traces of nucleogemmæ have ever been observed, and also because I believe I have been able to trace step by step the origin of the test-cells from the surrounding follicular zone.

THE MARINE BIOLOGICAL LABORATORY, WOOD'S HOLL,

July 15, 1890.

DESCRIPTION OF PLATE VIII.

[All the figures are camera drawings. Figs. 1, 2, 3, and 6 inclusive are from preparations hardened in Kleinenberg's picro-sulphuric and stained in borax-carmin. Identical preparations were obtained with Davidoff's acetic-sublimate and acetic-picric. Figs. 4 and 5 were hardened in acetic-picric and stained in borax-carmin. Figs. 11-17 were prepared in picro-nitric acid, and I must express my obligation to Dr. H. V. Wilson, who supplemented my now imperfect material from some that he had carefully preserved in the Bahamas. Figs. 7, 8, 9 and 10 are from preparations killed in weak osmic, stained in silver nitrate, etc., and cut in paraffin.]

Figs. 1, 2, 3, and 6 are sections of ova of *Cynthia ocellata*. Fig. 1, Zeiss 4 f.; Figs. 2, 3, 6, Zeiss 2 f.

Figs. 4 and 5 are sections of ova of *Cynthia partita*. Zeiss 2 f.

Figs. 7, 8, 9, 10 are from *Cynthia partita*; Figs. 7 and 8, surface views of follicle cells; Figs. 9 and 10, cross-section of egg. Zeiss 2 f.

Figs. 11-17 inclusive are sections of ova of *Clavellina*. Zeiss 2 f.





THE ORIGIN OF THE MESOBLAST-BANDS IN ANNELIDS.

EDMUND B. WILSON.

I.

SINCE the publication of Salensky's memoirs on the formation of the germ-layers in various Polychæta (*Psygmobranchus*, *Nereis*, *Pileolaria*, *Terebella*, *Aricia*)¹ it has been generally accepted that the mesoblast-bands ("secondary mesoblast") of annelids arise in some cases by direct proliferation from the ventral ectoblast, and often without the agency of pole-cells, or teloblasts ("primary mesoblasts, or pro-mesoblasts"). "Par-tout," he says, "où j'ai réussi à observer les jeunes stades de l'évolution du mésoderme somatique, ce feuillet, à son début, consistait en un épaississement ectodermique qui, sous forme de deux bandelettes, règne suivant l'axe longitudinal du corps; ce n'est que dans le cours du développement qu'il se sépare de l'ectoderme."² This result was in harmony with Kleinenberg's earlier studies upon *Lumbricus*;³ it received, apparently, a complete confirmation through the same author's splendid study of *Lopadorhynchus*,⁴ a work that seemed at the time to place the ectoblastic origin of the entire mesoblast in a number of the marine annelids beyond the possibility of doubt.

Yet this conclusion in one sense only served to render the general subject of mesoblast-formation in annelids less intelligible than ever. It had been demonstrated by Kowalevsky,⁵ Hatschek,⁶ and Whitman,⁷ that in some cases the mesoblast first appears in the form of a pair of large cells (teloblasts), by

¹ *Arch. de Biol.*, III., IV., VI.

² *Arch. de Biol.*, VI., p. 618.

³ *Quart. Jour. Mic. Sci.*, XIX., 1879.

⁴ *Zeitsch. f. wiss. Zool.*, XLIV., 1886.

⁵ *Mém. Acad. Imp. St. Petersburg*, XVI., 1871.

⁶ *Arb. Zool. Inst. Wien.*, I., 1878.

⁷ *Quart. Jour. Mic. Sci.*, XVIII., 1878.

the proliferation of which the paired mesoblastic bands are produced. The teloblasts are often differentiated at a very early period, — sometimes even prior to the gastrulation, — arising near the region corresponding to the posterior lip of the blastopore. In no case do they arise from the ectoblast; in some cases they seem to arise from, or at least to be closely associated with, the cells of the archenteron. In still another class of cases the mesoblast appears to arise neither by delamination from the ectoblast, nor from teloblasts, but from a central mass of “mes-entoblast,” the lateral portions of which give rise to the mesoblast-bands, and the central portion to the entoblast. This would seem to be the case, for instance, in the development of *Enchytræoides* as described by Roule;¹ and it is apparently the case also in *Spirorbis*, according to the investigations of my former pupil, Miss H. Randolph, whose preparations I have carefully examined.²

How are these various modes of mesoblast-formation to be reconciled? It is impossible to doubt the homology of the mesoblastic bands in *Polygordius*, *Eupomatus*, *Lumbricus*, *Clepsine*, *Lopadorhynchus*, and *Enchytræoides* — a series that includes representatives of the three modes of mesoblast-formation I have mentioned. It must, therefore, be possible to reduce these modes of development to a common type, and it is a remarkable illustration of the elementary state of our knowledge of annelid development that no one, as far as I am aware, has made even a suggestion as to how this is to be done. The only exception known to me is an hypothesis put forward in my recent paper

¹ *Ann. d. Sci. Nat.*, VII., 1889.

² Götte has described the mesoblast of *Spirorbis nautiloides* as arising from a pair of large teloblasts, derived from the archenteron. This, however, is certainly a blunder. As Salensky has suggested, and Miss Randolph has conclusively proved, the rounded body, mistaken by Götte for a pair of primary mesoblasts, is simply the rounded, hollow posterior part of the mesenteron. The mesoblast first appears in the form of two lateral masses which join each other posteriorly, and in some cases seem to fuse with the walls of the mesenteron. Salensky describes these masses as delaminating from the ectoblast in *Pileolaria*, a form nearly related to *Spirorbis*. In the latter form, however, the mesoblast-cells are quite distinct from the ectoblast from the earliest period at which they are distinguishable, while they are so intimately related with the entoblast, as to form with it, apparently, a common mass of mes-entoblast, as in *Enchytræoides*. Miss Randolph's researches are, however, still incomplete, and a more thorough study of the earlier stages may lead to a different interpretation.

on *Lumbricus*¹—a somewhat unsatisfactory suggestion, which, however, had the merit of emphasizing the importance of a careful study of the relations between the germ-bands and the blastopore in the Polychæta. During the past two years I have investigated several forms, with this end in view, in the hope of finding facts that might bring into some relation the teloblastic origin of the mesoblast (represented by *Clepsine*, etc.) and the delaminate mode of origin (*Lopadorhynchus*), but until recently the puzzle remained as great as ever. During the past summer, however, I was fortunate enough, through the kindness of Dr. E. A. Andrews, to procure very abundant material for the study of the early stages of two species of *Nereis* (*N. limbata*, Ehlers, and *N. megalops*, Verrill), and the facts thus brought to light point the way, as I believe, to a solution of the problem. The interest of the results depends largely upon the completeness with which the early stages can be followed in detail; and this in turn is owing to the extraordinarily favorable character of the eggs for observation. They are transparent, of comparatively large size, and they may be procured in abundance. The four primary entoblast cells (which are the remnants of the four macromeres of the typical eight-cell stage) remain undivided until a very late stage; *i.e.* until the trochophore form is attained and the eyes and mouth have appeared. Thus the axes of the embryo may be located with the greatest precision throughout the entire process of cleavage and gastrulation; and the certainty of the orientation is increased by the following circumstance. The transparent macromeres contain large oil-drops, which run together during the development, until, in the great majority of cases, only four are left (one in each macromere), *viz.*: a pair of smaller and a pair of larger drops, in correspondence with the size of the macromeres in which they are respectively contained. The smaller pair mark the anterior extremity, the larger the posterior, and they are bilaterally arranged on either side the middle line (*cf.* Fig. 6). There are other advantages, equally great, that facilitate the precise study of the early stages, but these will be described hereafter.

Aided by these favorable conditions, I have been able to trace the origin of the mesoblast-bands from the beginning of development. As in *Lopadorhynchus* and similar types, the

¹ *Jour. Morphology*, III., 1889.

trochophore *seems* to consist at first of two layers only. The mesoblast, like the neural foundations and those of the seta-sacs, arises directly from a thickened bilobed ventral plate; *i.e.* it seems to arise from the ectoblast. But a further examination of the matter gives it a different aspect. The cells of the ventral plate differ markedly from the remaining cells of the outer layer. They are larger, differently granulated, and upon treatment with certain reagents (combinations of acetic acid, etc.), assume a brownish color that differentiates them very sharply from those of the upper pole. This renders it possible to trace

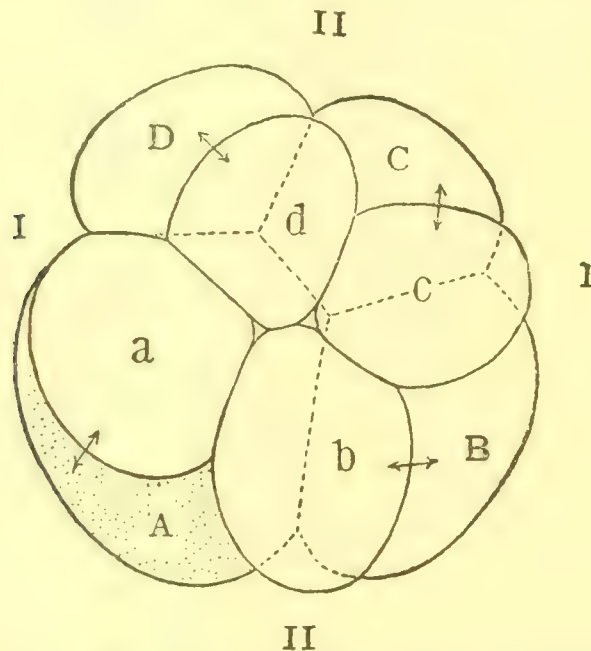


FIG. 1. — Eight-cell stage of *Nereis* viewed from the upper pole. I.-I., the first cleavage-plane; II.-II., the second; A, B, C, D, the four macromeres; a, b, c, d, the corresponding micromeres.¹

their origin, cell by cell, from the beginning of development, and this origin is such as to show that the mesoblast is completely segregated in the anterior part of the plate, while the posterior part alone gives rise to ectoblastic structures (neural plates, seta-sacs). Moreover, *each of the two divisions of the ventral plate may be traced back to a single cell (pro-teloblast), which is obviously homologous to a corresponding cell in the early embryo of Clepsine.* A detailed account of the cleavage will be given in a forthcoming paper, but the following synopsis will suffice for the present purpose.

¹ All of the cuts are from camera drawings, and are not schematized in outline, though slightly simplified.

I have followed the cleavage process many times and find it to be extremely constant, the variations being so insignificant as to have no effect on its general character. The first cleavage divides the ovum into a larger (AB) and a smaller (CD) segment; the second divides CD into equal parts, C and D , and AB into unequal parts, of which A is the largest of the four, and B is intermediate in size between it and the two smaller segments, C and D . The third or equatorial cleavage separates four micromeres (a, b, c, d) at the upper pole from four macromeres (A, B, C, D , respectively, Fig. 1). The later development shows

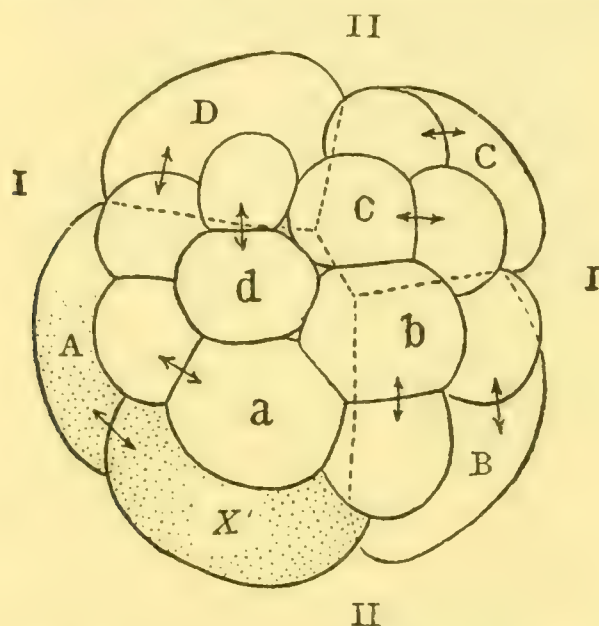


FIG. 2.—View from the upper pole of the sixteen-cell stage of *Nereis*. X , the first pro-teloblast which has been separated from A .

that the second cleavage-plane (II.-II.) corresponds precisely to the future median vertical plane of the adult body; C and D are the anterior macromeres; A and B , the posterior. The embryo is, therefore, not bilaterally symmetrical, from any point of view. Bilateral symmetry is, however, soon to be established.

At the fourth cleavage (Fig. 2) the four micromeres divide, not quite equally, and three new micromeres are formed from B , C , and D , respectively. A , at the same time, separates off a large characteristically granulated cell (X), which I shall call the first pro-teloblast. The regularity of the cleavage now ceases. A little later A again divides, separating off a second cell (Y), somewhat smaller than X , but with a similar granulation of the protoplasm. This I shall call the second pro-

teloblast. It lies between *A* and *X*, anterior, ventral, and somewhat to the left hand of the latter (Fig. 3). *A* is now approximately of the same size as *B*, and the embryo is bilaterally symmetrical, though not absolutely so, since both the pro-teloblasts are slightly displaced to the left, *Y* somewhat more so than *X*.

The gastrulation is strictly epibolic, the posterior lip of the blastopore is formed by the progeny of *Y*, the second pro-

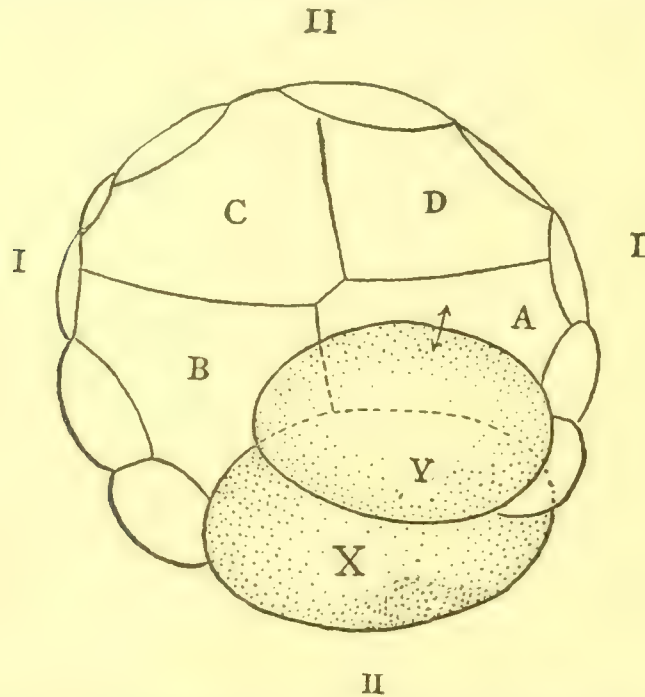


FIG. 3. — View of a later stage from the lower pole, showing the two pro-teloblasts, *X* and *Y*, the four macromeres, and the edge of the cap of micromeres.

teloblast, and the stomodæum is developed from the micromeres that close in the blastopore.

The principal interest of the development, from our present point of view, centres in the fate of the pro-teloblasts. *From these two cells the entire ventral plate arises, its anterior cells from Y, its posterior cells from X. From Y arise the mesoblast-bands, from X the neural plates, the seta-sacs, and other structures still undetermined.* *Y* first divides into two equal cells (the primary mesoblasts), and *X* soon does the same, first, however, invariably separating two smaller cells (Fig. 4). The bilateral symmetry of the embryo is now conspicuous, though the slight displacement of the teloblasts to the left still remains. Each of the primary mesoblasts now separates off a somewhat smaller cell at its outer side, thus forming a transverse series of four

cells which lie at the surface and form the posterior lip of the blastopore. Each of the *X*'s now separates off a smaller cell

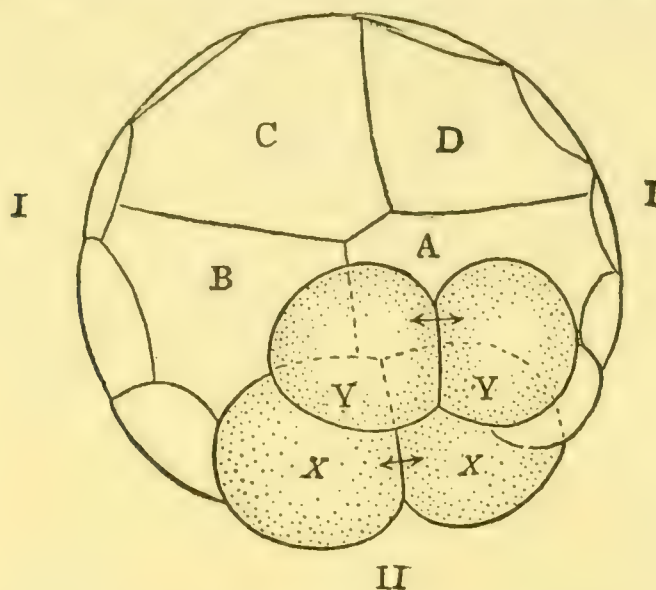


FIG. 4. — View corresponding to that given in Fig. 3, after the division of the protoblasts. *Y, Y*, the primary mesoblasts; *X, X*, the two primary posterior teloblasts.

in front, then a small cell at its outer side, and finally divides longitudinally into two equal parts. At this period (Fig. 5) the

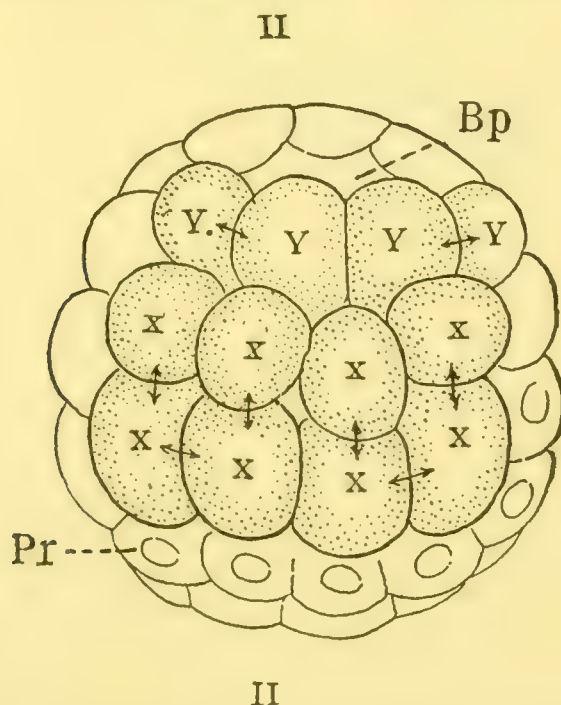


FIG. 5. — View from the ventral side of a still later *Nereis* embryo. The ventral plate consists of fourteen cells (two small lateral cells are not shown). *Bp*, the blastopore; *Pr*, micromeres from which the prototroch is almost immediately afterwards developed.

ventral plate consists of twelve principal cells, arranged in two longitudinal rows of three each on each side of the median line. Anteriorly are the two primary mesoblasts with their first-formed derivatives (*Y-Y*); posteriorly are four teloblasts (*X-X*), and four smaller cells derived from them, as shown in the figure.

In the next stage the four smaller *X*'s divide obliquely, while four smaller cells are produced anteriorly from the four larger *X*'s. The four *Y*'s are now rapidly pushed into the interior, being overgrown by the progeny of *X*, which advance upon them from behind (Fig. 6), and by the micromeres advancing from the

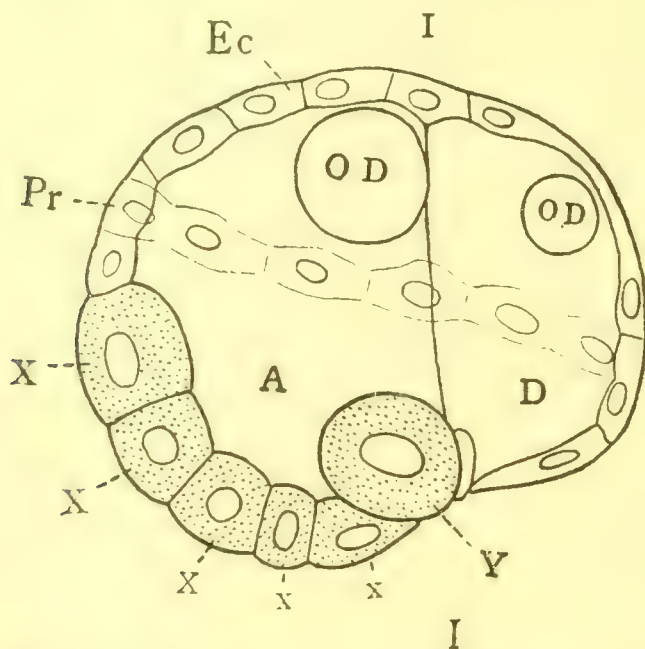


FIG. 6.—View of a still later stage in optical longitudinal section from the right-hand side. I.-I., the first cleavage-plane; *Ec*, the ectoblast derived from the micromeres; *Pr*, the prototroch; *OD*, the oil-globules; *Y*, primary mesoblast in process of pushing in; *X-X*, progeny of the first pro-teloblast. The blastopore, which was situated just anterior to *Y*, has been closed in by the micromeres.

sides and in front. As they are pushed in, the smaller *Y*'s divide, and a second pair of smaller cells are separated from the two large *Y*'s (primary mesoblasts), which are now scarcely larger than the cells to which they have given rise, or than the superficial cells (descendants of *X*) among which they are imbedded. After the next division the teloblasts become indistinguishable, and the mesoblast-bands seem to fuse with the ventral plate; so that an examination of the embryo at this period, without a knowledge of its earlier history, would certainly lead to the conclusion that the mesoblast-bands arise by proliferation from

the ventral ectoblast, as Salensky describes it. From their point of origin the bands, each now represented by a group of six or seven cells, extend upwards between the outer layer and the four entoblast-cells, along the line of contact of the two anterior cells (*C*, *D*) and the two posterior (*A*, *B*). Their later history will be described elsewhere.

Let us now return to the progeny of *X*. Increasing rapidly in number, both by their own divisions and by the addition of cells formed from the four posterior teloblasts, they give rise to a broad, bilobed plate, consisting throughout of a single layer of granular cells, and occupying the greater part of the lower half of the embryo. The prototroch is developed from a series of micromeres, at first single, that encircles the equatorial belt of the embryo, and lies immediately behind the four posterior teloblasts. The latter persist for a considerable period, but ultimately disappear. The two outer ones first break up into smaller cells, and as this takes place, the remaining two separate from each other along the median line. Thus the ventral plate becomes bilobed behind, with a V-shaped area between the two lobes, and a single teloblast at the tip of each. This teloblast remains until each half of the ventral plate contains fifty or more cells, still lying quite at the surface. Ultimately it disappears, and the proctodæum is formed in the anterior part of the V-shaped area. At a still later period the ventral plate thickens, becoming several layers deep on each side the median line, and gives rise to the neural plates and the seta-sacs. Its relation to the nephridia and the circular muscles is still under investigation.

Let us now compare these facts with the development of *Clepsine* and *Lopadorhynchus*. In *Clepsine* the large posterior macromere first separates off a single micromere (as in *Nereis*), and then divides into two large cells. The upper right-hand cell ("neuro-nephroblast" of Whitman) has precisely the same relation to the rest of the embryo as the first pro-teloblast, *X*, of *Nereis*. In *Clepsine* this cell breaks up into eight teloblasts, viz. (still using Whitman's terminology): two neuroblasts, four nephroblasts, and two lateral teloblasts; and these give rise to all the structures of the ventral body-wall excepting those arising from the mesoblast-bands; *i.e.* the neural foundations, a part of the nephridia, some of the ventral ectoblast (probably), and

perhaps to other structures.¹ In *Nereis* the corresponding cell breaks up into four instead of eight teloblasts, which give rise likewise to those parts of the ventral body-wall not derived from the mesoblast-bands; *i.e.* the neural plates, the ventral ectoblast, the seta-sacs, and probably other structures (for the present I leave the nephridia and the circular muscles out of consideration). The foregoing considerations render it practically certain that *the first pro-teloblast of Nereis (X) is the homologue of the "neuro-nephroblast" of Clepsine.* In *Clepsine* the second and larger cell produced by the division of the large posterior macromere (which I shall call the common primary mesoblast) divides into equal parts, which become the primary mesoblasts, and give rise to the mesoblast-bands, in the usual manner. *The second pro-teloblast (Y) of Nereis is therefore the homologue of the common primary mesoblast of Clepsine.* It therefore appears that *Clepsine* and *Nereis* agree in every essential feature of development. They differ only in secondary details, — in the ultimate number and arrangement of the teloblasts, and in the fact that they and their products are at first superficial and form a part of the outer layer, — falsely called the ectoblast. It is interesting to recall in this connection the fact that in *Lumbricus* eight of the ten teloblasts likewise remain for a long time at the surface.

Turning now to *Lopadorhynchus*, we find here also a bilobed ventral plate, as in *Nereis*, but no teloblasts, *in the earliest stages thus far observed.* From it arise, as in *Nereis*, the mesoblast-bands, the neural plates, and the seta-sacs; and I think the ventral plate must be regarded as completely homologous in the two forms. They differ only in the earlier segregation and differentiation of the mesoblastic material in *Nereis* which leads to the formation of a pair of transitory teloblasts, which, however, form part of the ventral plate. Meyer makes the interesting statement in a recent paper² that he has satisfied himself that the neural elements and the mesoblast-bands do not arise in *Lopadorhynchus* from a common foundation, but from "Zwei verschiedene, nur dicht zusammengedrückte Bildungsheerde."

¹ Bergh asserts that the lateral teloblasts and the nephroblasts give rise, in *Lumbricus*, to circular muscles, and are therefore myoblasts. I shall return to this question in a later paper.

² *Biol. Centralblatt*, 1 Juli, 1890.

This is precisely what occurs in *Nereis*. The term "ectoblast" as applied to the ventral plate as a whole is, however, a misnomer. Its cells form rather a still undifferentiated tissue which is nowise to be regarded in the same light as the ectoblast of the upper pole.

II.

It now becomes an interesting question whether the secondary mesoblast of annelids can be shown in all cases to arise from a single pair of teloblasts; for the case of *Nereis* shows that they may be present only in very early stages, so as easily to be overlooked. The entire subject demands re-investigation from this point of view. I have carefully studied the development of *Hydroides dianthus* (a form nearly allied to *Eupomatus*), by following the cleavage of the living ovum, by examination of stained and cleared embryos, and by actual sections. The cleavage is in every detail identical with that of *Eupomatus*, the gastrulation takes place in essentially the same manner, and the trochophore is of quite the same type. Yet I have been unable to identify the teloblasts at any period. They are certainly not present at a stage when the mesoblast-bands consist of not more than five or six cells each (*cf.* Hatschek's Figs. 43 to 46).¹ At this period each band ends posteriorly in a group of about three cells, two of which, not perceptibly larger than the others, are joined by a narrow bridge of protoplasm stretching across in the angle between the proctodæum and the wall of the anal vesicle. The head-kidney lies outside the mesoblast-band, and is only connected with it at its anterior end.²

In its earliest recognizable condition (*cf.* Hatschek's Fig. 33) the mesoblast-band consists of a group of three or four cells

¹ Arb. a. d. Zool. Inst. Wien., VI., 1885.

² I have been able, by the study of these embryos, to establish the interesting fact that the head-kidney opens posteriorly into the proctodæum. Under a high power (Zeiss Apochromatic Homogeneous, 3 mm.) the canal can easily be followed from its beginning near the front end of the organ, along the outer dorsal border of the latter, into the antero-lateral part of the proctodæum. This can be done most readily during the activity of the cilia (which is intermittent); but the canal can easily be followed in the quiescent state, in both the side and ventral views of the larva. This fact seems to remove all doubt of the homology of the trochophoran head-kidney with the nephridia of the Rotifera. I shall return to this point at another time.

wedged into the angle between the posterior part of the archenteron and the ectoblast: no one of them can be identified as the teloblast. Besides this "secondary mesoblast" there are at this period a number of scattered cells, lying in the narrow cleavage-space. Some of these are applied to the ectoblast, some to the entoblast, where they form a contractile network surrounding the archenteron; some stretch across the cleavage-cavity. These cells obviously represent a part of the "primary mesoblast" or "mesenchyme," the relation of which to the secondary mesoblast of the germ-bands has become one of the most interesting questions of annelid embryology. My observations on *Hydroides* indicate that the primary mesoblast arises in a manner similar to the process in *Polygordius*, as described by Repiachoff¹ and Metschnikoff,² though I am not yet able to give decisive evidence, in spite of repeated examination of the question. According to these authors the primary mesoblast arises in the form of isolated cells that take their origin "höchst wahrscheinlich," in the entoblast, at or just before the time of gastrulation. Metschnikoff makes no special mention of the formation of the secondary mesoblast-bands; but his general account implies, and his figures bear out this interpretation as far as they go, that there is no real distinction between the primary and secondary mesoblast, the latter arising in exactly the same manner as the former. For the present I am obliged to believe that the primary and secondary mesoblast have the same relation in *Hydroides*. The mesenchyme cells are of all shapes,—branching, elongate, rounded,—and appear to graduate both in form and in position into those of the germ-bands. The latter appear about the time of gastrulation, as two bilateral masses of cells that are pushed into the cleavage-cavity near the blastopore. Some of these cells appear to pass forwards and give rise to the "mesenchyme"; the remainder form the "secondary" mesoblast-bands, which at first are posteriorly at the sides of the proctodæum, but afterwards come into connection by a bridge that is developed on the ventral (anterior) side of that structure.

Through the courtesy of Mr. Agassiz I have had an opportunity to investigate *Polygordius*, the larvæ of which are very

¹ Zool. Anzeiger, 1881.

² Z. f. W. Zool., XXXVII., 1882.

abundant at Newport. In the very large series of larvæ in my possession all stages are represented from the adult condition¹ down to the youngest described by Hatschek and Fraipont. The species is closely similar to *P. neapolitanum* as described by Fraipont. An examination of the mesoblast-bands in these larvæ showed, to my extreme surprise, that *no teloblasts of any kind are present*, even in the youngest stages. The mesoblast-bands end behind, as in *Hydroides*, in a group of two or three cells, which are somewhat larger than those in front, it is true, but have none of the characteristics of teloblasts. Only in a single case have I found at the tip of the band a cell distinctly larger than the others, and this was on one side only. Is this absence of the pole-cells peculiar to the American species of *Polygordius*? Possibly; yet there is a fact that forces me to suspect the possibility of error on the part of Hatschek and Fraipont, improbable as such a suspicion may appear. If the germ-bands of a young larva be viewed *en face* from the ventral side in suitably prepared specimens, a very clear picture of the germ-bands will be seen, closely similar to that represented in Hatschek's Fig. 57. At the tip of each germ-band is a large, rounded, clear cell, meeting its fellow in the median line just in front of the anus, and to all appearances a "primary mesoblast." For such I at first mistook these cells, but, to my great surprise, sections in the various planes all agreed in showing that they formed part of the ectoblast, lay at the surface of the body, and had no connection whatever with the mesoblast-bands, though the latter end just below them. These cells are clear, vacuolated, with minute nuclei, and are undoubtedly homologous with the anal vesicles or anal glands of other annelid trochophores; though as far as I am aware they are here described for the first time. They have no connection with the paratroch.

¹ I have repeatedly observed the sudden metamorphosis of the last larval stage into the adult, which has been briefly mentioned by Kleinenberg. The cells of both the prototroch and paratroch are suddenly thrown off, and continue to swim for some time after their complete separation from the body of the young worm. The prototrochal cells are often thrown off, over the head, in the form of a girdle, which in some cases—I have observed it at least four or five times—is swallowed and digested by the animal. This recalls the curious habit of *Actinotrocha*, which at its metamorphosis throws off and swallows the greater part of the præ-oral lobe (Caldwell). This metamorphosis certainly throws an interesting light on the origin of such a mode of development as that of *Pilidium*.

I hesitate to suggest that two such experienced observers as Hatschek and Fraipont can have fallen into error on this point, yet it is a singular fact that neither of them figures or refers to the conspicuous præ-anal gland-cells, while both describe a large mesoblast-cell in exactly the same position, but lying below the surface. Each gives a single figure of the "primary mesoblasts" in cross-section, lying in the cleavage-cavity. But Hatschek gives also, in the same series of figures, exceedingly definite representations of "die fötalen Längskanälen," which, as Meyer and Fraipont have shown, and as I have convinced myself from the study of many series of sections, have no existence in nature. Fraipont's figure is scarcely more satisfactory. The minute nuclei and clear protoplasm of his figure have no resemblance to the large, conspicuous nuclei and granular protoplasm of teloblasts; they at once suggest the small nuclei and vacuolated cell-body of the "anal gland-cells." A closely similar view might easily be had of a rather thick, slightly oblique section, the "gland-cells" being tangentially cut, and appearing to lie inside the ectoblast on account of the sharp curvature of the surface where they lie. And in view of these facts I venture to suggest the desirability of a re-examination of the mesoblast-bands in the European species of *Polygordius*.

The non-existence of the teloblasts in later stages, which is apparently the rule among the Polychæta and (?) Archiannelids, by no means, however, affords any presumption that they are not present in earlier stages. The case of *Nereis* shows that it will not be safe to assume the absence of teloblasts without following the development, cell by cell, from the very beginning, and that wherever it is possible to make such a detailed study, we may pretty confidently expect to find teloblasts. There is, I believe, every reason to believe that in *Hydroides*, for example, the two groups of mesoblast-cells take origin in two cells, though they may appear at so early a period and differ so little in appearance from the adjacent cells as to elude any but the most searching examination. And it does not seem very rash to predict that the secondary mesoblast-bands, even of *Lopadorhynchus*, will yet be shown to arise by teloblastic development.

While the foregoing article was in process of publication I received R. S. Bergh's full paper on the germ-bands of *Lumbricus* [*Zeitschr. f. wiss. Zool.*, L. 3, 1890]. This admirable work contains a complete confirmation of my discovery of the teloblasts and cell-rows of the middle stratum of the germ-bands in Oligochaeta—a confirmation which has especial significance on account of the curiously exaggerated and, to me, quite inexplicable, hostility with which its author saw fit to receive the original announcement of that discovery. Dr. Bergh's paper, as a matter of course, exhibits in some degree the well-known and characteristic skill of its author in belittling the work of other investigators, but I observe with interest many signs of progress, both in knowledge of the germ-bands and in acquaintance with the usual forms of courtesy in scientific discussion. The author, for instance, no longer seeks to discredit my work by innuendoes directed against my scientific good faith; indeed, the existence of the teloblasts and cell-rows has even become "sehr leicht zu konstatiren"—an evidence of progress on which I congratulate my learned and courteous critic. It is not improbable that Dr. Bergh may in time be able to observe with equal ease the teloblasts and other structures I have described in *Nereis*.

Apart from the development of the glandular portions of the nephridia, my general account of the structure, mode of growth, and relations of the various parts of the germ-bands in *Lumbricus* is confirmed in every respect. He adds, however, the very interesting discovery that the products of the neuroblasts are reinforced by a median nerve-plexus that is taken up into the ventral nerve-chain between the two neural cords. His account of the "nephroblasts" and "lateral teloblasts" differs entirely from that given by Whitman and myself. Their connection with the nephridia is denied, and they are asserted to be "myoblasts" which give rise to the circular muscles. This result, if well founded, is of the greatest interest, and marks an important advance in our knowledge of annelid embryology. It is to be hoped, however, that the question may be carefully re-examined, by some fair-minded observer, in *Clepsine* or some other favorable form.

BRYN MAWR, PA.

CONCERNING VERTEBRATE CEPHALOGENESIS.

HOWARD AYERS.

IN this short resumé of some of the results of my investigations in vertebrate cephalogenesis, I shall not give a complete account of the morphological data upon which the conclusions are based ; but shall introduce only such facts as seem necessary for this presentation.

A more detailed account with a critical consideration of the literature bearing upon the subject is reserved for a more extended and illustrated publication which I have in preparation.

The problem of the origin of the vertebrate head, more especially the brain, from the invertebrate type is, so far as our knowledge yet reaches, an insoluble one ; but given the vertebrate type of body and central nervous system, we are in position to clearly demonstrate its phylogenetic outcome as represented in the mammalian head and brain.

Starting with the central nervous system of *Amphioxus*, we have to deal with an organ which affords many points of contrast with the axial nervous system of higher vertebrates and which serves, as some authorities think, to bridge over the chasm between the invertebrate type (arthropod and annelid) and the vertebrate.

With the exception of an undetermined small number of anterior segments, the nerve cord of *Amphioxus* is divided up into a series of physiologically equal segments as Steiner has shown ; but I feel confident from anatomical facts that further and more detailed experimentation will show that there are some modifications to be introduced into Steiner's results, and that there are differentiations physiologically which the crude methods of experiment used by him excluded from the physiological reaction. Steiner's results on other vertebrates would lead to a conclusion differing from that which he has published with regard to *Amphioxus*. For in his experiments on *Amphioxus*

he was unable to take into account any of the higher sense organs. Of course their relation to locomotion and the central mechanism effecting this relation entirely escaped him.

I hope to show that there are certain ganglionic centres in the anterior end of the nerve cord of *Amphioxus*, which compel us to call this region a brain strictly comparable with that of other vertebrates; for in this region we have the centres of special sense brought into relation with locomotion.

In my final paper I shall give an historical review of the ideas that have been held concerning vertebrate cephalogenesis. For it is in the gradual evolution of these ideas that we have the firmest support, and completest precedent, for leaving the prevailing ideas for those that can be shown to come nearer the truth in nature. The idea that the human skull (as typical of the highest vertebrates and hence erroneously thought to most certainly present genuine vertebrate conditions) was formed of relatively slightly modified vertebral bodies and their processess, had its eminent exponents and ran its course. It was succeeded by the theory that it was only in the cartilaginous cranium that remnants of primitive segments were to be sought. This form of solution was modified by the statement that only a portion of the primordial cranium could possibly show traces of the primitive segmented character.

This brings me to the expression of the view, which has firm support in facts, that the primordial cranium is never influenced by vertebral segmentation, as it appears in, and belongs to, a stage antecedent to the formation of vertebræ; further, that it is not primarily influenced by the mesomeric segmentation of the body, since it arose at a later period phylogenetically and after the mesomery ontogenetically. The primordial cranium has been gradually acquired by vertebrates, and its rudiments were developed first in or near the horizontal plane in which the chorda lies. Extending in a direction more or less parallel to the chorda, it does not necessarily show traces of the primitive segmentation of that part of the body in which it is developed, since many, at least, of the features of segmentation, had long vanished before a protecting cranium formed about the nervous axis. In such a structure we would expect cœnogenetic (ontogenetic) variations to be of frequent occurrence.

What I have said above does not exclude the possibility of

the inclusion of vertebral remains within the skull, nor is this view influenced by the fact that such inclusions are known to exist, *e.g.* in the cartilaginous fishes, etc., and for this reason; the skull is formed about the brain and includes various organs, not always the same, within its *substance*. As illustrations of the variations met with, we have in some cases the aorta included in the cartilaginous basis cranii, though usually it is left out, the notochord usually for the whole of its cranial length, though it may be left out in part, as for example its anterior end may lie below the cartilage or above, *i.e.* inside the cranial cavity either projecting beyond the pituitary prominence or running out on the inner face of the skull behind it. There are other facts of this class which have not received enough attention.

A. *The anterior end of the neural axis of Amphioxus is a brain, and corresponds with a certain definite portion of the brains of other vertebrates. Its anterior wall is the homologue of the lamina terminalis of other vertebrate brains, and the anterior portion of its unpaired ventricle is the thalamocœle. There is a posterior portion of the ventricle intimately associated with a ganglionic tract, which corresponds to the mesocœle, while the myelocœle remains, more or less widened, varying much in different individuals, but always in an undifferentiated condition.*

Although the nervous system of *Amphioxus* has been oftentimes studied, many features of importance remain to be described, and the well-established relations deserve reconsideration in connection with the new ones. With the improvements in methods of preparation, results unobtainable otherwise are reached, which show that we may still hope for more light on important questions from even well-worked fields. The preparations I have studied were made by the celloidin method of imbedding and nitric acid maceration process, and I can recommend both as giving excellent results.

I may say, to begin with, that I do not consider *Amphioxus* to be a degraded form, in the usual sense of that word as employed in vertebrate phylogeny. Its larval modifications cannot be called *degradations* of structure, nor are its adult peculiarities to be looked upon in the light of degraded or bizarre modifications of *normal* vertebrate conditions; on the contrary, they are to be regarded as not only *normal*, but also

as *primitive*, and in a certain degree *ancestral*, conditions of structure.

The derivation of the vertebrate phylum from some simple type closely approaching *Amphioxus*, in detail of structure, in almost¹ all its organs, as we know them at the present time, becomes a morphological necessity. Instead of precluding the possibility of establishing the claims of *Amphioxus* to such position in the phylum, every advance in our knowledge of the life-history of *Amphioxus* serves only to strengthen such claims.

It is to be regretted that certain zoölogists should have so dulled their morphological sense as to be found denying to *Amphioxus* any position whatsoever among vertebrates. As an offset to such rash opinions, I will only quote the words of Professor Huxley, giving his conclusions after a careful study of the anatomy of *Amphioxus*, and a detailed comparison with other fish forms: "In all other respects, however, it conforms (except in the absence of auditory organs) to the vertebrate type; and considering its resemblance to the early stages of *Petromyzon*, . . . I can see no reason for removing it from the class of *Pisces*." I might quote other eminent authority in favor of the strictly vertebrate nature of *Amphioxus*, but I think this should suffice. Surely we may never hope to know more of the mystery of the origin of vertebrates by ignoring the most important of the still accessible forms that lead us back to the beginning of our type.

It has become necessary, in any discussion of the homologies of the *vertebrate brain*, to define more accurately what is to be understood by the term "*vertebrate brain*." Evidently the definition of the word must include the names and relations, as far as lies within the province of a definition, of all those parts common to all vertebrate forms, excluding from the principal sentence of the definition all exceptions however important. We have not lacked for definitions more or less specific, but until quite recently I do not know of any investigator who has attempted to define the organ in terms harmonious with the

¹ July 27. I have just seen Boveri's paper, "*Über die Niere des Amphioxus*"; and since this investigator has shown in most conclusive manner by his important discovery, that the kidneys of *Amphioxus* are ancestral structures, I am ready to withdraw the limiting word "almost," since it was mainly with reference to the kidney system that the reservation was made.

present condition of morphology and physiology. Steiner has shown, by a beautiful series of experiments on numerous forms, that we may only speak of a brain from the physiologist's standpoint when we have associated with the general centre of locomotion one or more of the organs of the higher senses, and this definition is entirely admissible from a morphological standpoint. While Steiner's experiments do not show the presence of a brain in *Amphioxus*, they do not, I think, in any way demonstrate or even render probable its *absence*, and it can be shown that the morphological or physical basis required by Steiner's definition is present.

I would define the vertebrate brain as follows: *The "vertebrate brain" is that portion of the anterior part of the axial nerve cord, associated with organs of special sense, containing an enlargement of the central canal which is carried out into all structures formed by the outgrowth of the brain wall. Its walls contain the principal centres for the co-ordination of sensations and movements. All further additions to this simple brain (Amphioxus) are made in response to the demands of the organs of special sense, with which is associated extension of the co-ordination apparatus. With such additions we have the compound brain of all other known vertebrates up to man inclusive.*

Reasons why the anterior end of the nerve cord of *Amphioxus* is a brain. It is a brain because

1. It forms the anterior termination of the neural axis.
2. It stands in intimate relation to the sense organs eye and nose.
3. It gives off at least two pairs of sensory nerves provided with peripheral ganglia.
4. It possesses large groups of ganglion cells forming centres of co-ordination.
5. It possesses an enlarged section of the central canal in the form of an unpaired ventricle with three well marked diverticula, two optic, one olfactory.
6. It is the largest part of the nervous system, at a time when the massive musculature and branchial apparatus of the anterior middle fourth of the body has not reached the stage requiring much enlarged central accommodations.¹

¹ When, however, the innervation of the locomotive apparatus (which in the adult animal more than equals the remaining organs in bulk) is fully developed, the

7. It shows in young larvæ growth to such an extent as to cause a ventral flexure of the chorda, the brain itself bending downwards, thus producing a "cranial flexure."

8. It shows in all other details of structure that *it is not* simply the *anterior end of the spinal chord*, but a *brain*.

9. It shows in a larval stage soon after the differentiation of fibres in the neural axis (larvæ with one gill slit), a marked differentiation into ganglionic and fibrous regions, and the boundaries of the unpaired ventricle as well as the lamina terminalis are distinctly marked out. There is then a ventricular segment of the brain reserved for the special sense organs. The fibres appear simultaneously with the formation of the pigment spot, and are in all probability the ways by means of which the sensations from this special sense organ are conveyed backwards to the motor centres.

10. *Since Amphioxus is a vertebrate, these relations MUST have direct and important bearings on the phylogeny of the vertebrate brain and head, and will afford us invaluable aid in clearing up these intricate problems.*

B. *The large collections of ganglion cells just posterior to the thalamocœle are homologous with the medullary nuclei of other vertebrates, since their connections show them to be centres for the control of the branchial apparatus, and the sensory and motor structures lying in the territory of the gill basket,—e.g. centres of respiration, deglutition, etc.*

These groups of large ganglion cells do not reach quite to the thalamocœle. A portion of the wall bounding the ventricle posteriorly is free from them, and is made up of such cells as are strictly comparable with the cellular elements of the mid-brain of higher forms. From this narrow territory and the central canal contained in it is derived the mesencephalon and

brain is relatively smaller, but it still has its peculiar structure. We have other instances of the actual preponderance in size of circumscribed tracts of the nervous axis over the brain. In those ancient forms, *e.g.* *Stegosaurus*, whose remains have been described by Marsh and others, where the sacral canal is shown to greatly exceed the cranial cavity in its cubic dimensions. But here there is a doubt as to whether the nervous axis in the sacral canal really filled the space, or whether it bore proportionately the same relations to its cavity that the brain did to its cranial space. There is reason to seriously doubt that the sacral enlargement of the cord was actually larger and more important than the brain in relation to the locomotive mechanism of the animal.

mesocœle of higher forms. The cerebellum is simply a dorsal commissure of the medullary region, as has been shown by Osborne and others from studies on higher vertebrates. I have found no trace of a cerebellar commissure in *Amphioxus*. The open ventricle of the medulla is produced by the great increase of ganglion cells, and especially of the fibre tracts which must of necessity pass through this region in order to reach the centres of co-ordination in the parts anterior to it. The basal and lateral portions of its walls are thus greatly thickened, the ganglion cells of the nerve nuclei arrange themselves in harmony with these changed conditions, and the dorsal wall is greatly extended without at the same time being structurally complicated. The medulla of higher vertebrates is not co-extensive in all forms and includes a varying number of the segments represented in *Amphioxus* by simple spinal segments.

The brain of *Amphioxus* possesses all those functions which in higher vertebrates are possessed by thalamencephalon mid-brain, and medulla, except an undetermined number of the posterior segments of the medulla of the higher forms. Of course these functions are all of a milder nature than in the higher animals.

The reason for Steiner's failure to find a medullary centre of co-ordination lies in the intense nature of the stimuli applied to the nervous apparatus (resection, etc.) and to the small size of the region to which the stimuli must be applied. The nervous apparatus is of such a delicate nature that such strong stimuli serve to call forth the activities of each segmental centre of locomotion, and prevent the observation of any other results of stimulation.

Much more delicate methods must be used to obtain reaction of the organs of special sense. Of the possible means of experimentation, which appear to me likely to give good results in the case of the pigment spot and the olfactive pit, are circumscribed application of bundles of light rays of varying intensity and wave lengths, and the application of olfactive stimuli by means of fine capillary tubes to the resting animals in sea-water.

*C. The ontogenetic changes of the neural axis in other vertebrates carries the brain through the condition which in *Amphioxus* remains permanent as the adult brain.*

As has been demonstrated by several recent investigators, the fore-brain and olfactory lobes are simple outgrowths of the dorsal wall of the thalamencephalon. They appear early in the development, it is true, but such early appearance is undoubtedly a cœnogenetic phenomenon. That there is a substantial agreement between the *Amphioxus* brain, and the higher vertebrate brain of this stage, is perfectly evident. There is always a thalamocœle bounded anteriorly by a lamina terminalis to mark the boundary of the primitive end of the neural axis, and above, behind and on either side of this primitive lamina terminalis, the optic diverticula are given off from the brain.

D. *All the sense organs developed in connection with the anterior end of the Amphioxus body are probably paired; some of them certainly are, e.g. the eye-spot.*

As I shall show in the next paragraph, the eye of *Amphioxus* exhibits unmistakable traces of bilateral symmetry and a tendency to develop into two pigmented areas in connection with diverticula of the thalamocœle, so that here I need only mention the fact, that I have been able to trace fibres from the olfactory organ through the walls of the olfactive diverticulum or bulbus olfactorius to both sides of the brain. Whatever this organ may prove to be, on further investigation of its ontogeny it is supplied from *bilateral brain centres* and affects both *right and left co-ordinating tracts*. The organs of special sense developed in the course of the two anterior pairs of nerves are certainly bilaterally symmetrical structures.

E. *The eye-spot or eye of Amphioxus is the forerunner of the vertebrate eye, and shows traces of several stages in the development of the retina of higher forms. In itself it is not an organ of sight, but a light-perceiving organ.*

After a careful study of the *Amphioxus* eye-spot, and related structures, I have become convinced that this animal presents us with the earliest stage in the phylogenetic development of the vertebrate eye.

As is well known, this eye-spot, which is considered a pigment spot, lies across the anterior end of the neural axis in the anterior end or wall of the brain, *i.e.* in the lamina terminalis, in an extended sense. It is not widely known, however, that this

pigment spot assumes a large variety of shapes as well as positions, with respect to the anterior wall of the ventricle and to the first pair of cranial nerves. The most usual form is that of a slightly bilobed mass; the lobes being placed to the right and left of the median line, so as to cover the roots of the first pair of cranial nerves more or less completely.

Other forms have been described by the various authors who have written upon the subject, and they are easily observable in any series of individuals. As already stated, the spot is very variable in size and shape, and its extremes may be set, as the convex or concave (forwards) lens shape, and the close grouping of most of the pigment cells in the wall of the lamina terminalis on the one hand, and the separation of the mass into three portions (two ventral and lateral, and one dorsal and median) with outlying scattered pigment cells on the other hand. These various forms grade insensibly into each other, and evidently depend in large degree, if not entirely, upon the migratory capabilities of the pigment cells in a state of nature.

These variations, then, are caused by the shifting of the pigment cells. It may be frequently observed that the two pigment masses pass out into the two nerves of the first pair. Where this modification occurs, it will be found associated with a pair of diverticula of the median ventricle which make their way out into the nerve roots. These diverticula are lined by the same cells that form the inner lining of the median ventricle, and the pigment cells lie among the cells of the second layer, or layer of percipient elements.

In this manner the light-perceiving organs are carried toward the surface at the ends of diverticula of the primitive brain cavity, reproducing in the phylogeny of *Amphioxus* the first steps of the series known for the remaining vertebrates.

We have only to think of these two pigmented optic structures brought into relation with the ectodermic structures — lens, cornea, etc., — of the higher vertebrate eye, in order to have all the steps of eye production carried out in *Amphioxus*. We know of no such relation of the ectoderm in the *Amphioxus*, and consequently conclude that, all facts considered, *Amphioxus* is descended from some ancestral form along with other vertebrates, but that, owing to its simple habits of life, has never required a lens or other focusing apparatus for the pur-

pose of image perception, — simple light-perception sufficing for all its needs.

As Hatschek has shown, the pigment spot appears in the nervous layer long before the closure of the anterior neuropore. One of the causes operating to transfer the pigment from the brain wall to the periphery is the constant growth from the larva to the adult, and the consequent thickening and increasing opacity of the superjacent tissue.

After these diverticula have formed, there is left of the anterior brain wall between them a median portion which, as we shall see, is the homologue of the lamina terminalis of the remaining vertebrates.

The lamina terminalis of this stage contains the basis of the cerebral lobes, and the olfactive centres of all the higher forms.

The relation of the parts here described corresponds with the facts as given in the latest and most accurate studies of vertebrate ontogeny from all classes of vertebrates, which form a valuable basis for the examination of this proposition from the comparative standpoint.

An examination of Hatschek's figures, 64, 65, 67, and 69, for an explanation of the relation of the parts, shows that the most anterior portion of the neural plate of the young larva remains for a long time in its primitive flattened, uninclosed condition. Here the dorsal surface of the plate is still exposed to the direct action of external stimuli; its face is in or near the horizontal plane, and hence looks vertically, or nearly vertically, upwards. The eye-spot is developed in this area, and it clearly occupies the most favorable position for an important sense organ that the body of *Amphioxus* affords.

As the anterior neuropore closes up more and more, the anterior lip grows upward curving backwards, the pigment spot comes to lie in the vertical portion of the anterior end of the now completed canal; consequently it is strictly terminal in its adult relations, but may, and frequently does, assume a position at the side and behind the end of the axis.

For greater functional power, the central (median) portion of the pigment spot has grown upwards (dorsad), and carrying with it a portion of the ventricular wall has produced the pineal eye.

Let us follow through the development of the nerve cord of the larval *Amphioxus*. Formed as a thickened plate of cells on

the dorsal surface of the embryo, for a long time it lies open and exposed to the sea-water; its cells bear cilia which are doubtless sensitive to various stimuli. Long before the neural plate is converted into a cylinder (but only after the plate has been overgrown by the lip of the blastopore), pigment makes its appearance in some of the cells of the plate. At present we have no complete account of the appearance or early relations of this pigment matter; but the mass called the eye-spot appears shortly after the first mass which arises in the middle of the length of the plate, which at this stage falls in the fifth somite. The eye-spot soon becomes much more important than any of the posterior pigment-cell groups, and remains so throughout life. As already stated, it at first faces upward, but later acquires a facing at right angles to this direction on account of the closure of the neuropore, the development of the head fin, and the gradual thickening of the tissue directly above the spot. Of course with this thickening of the body walls, more and more light is cut off from the spot, and the direction from which light reaches the spot in greatest abundance is from the front and sides of the head. The head fin fold divides the light falling upon the spot into two lateral bundles of rays, which, owing to less thickness of tissue through which they are compelled to pass, fall upon the sides of the spot with greater energy than light from any other source.

It is, then, owing to the relations of the external features of the head that the primitively median pigment body (not necessarily from an unpaired source) divides into two lateral portions, each of which strives to get nearer the surface, and in accomplishing the transposition cause an outgrowth of the brain wall, producing the two antero-lateral diverticula spoken of above.

The relation of the two diverticula to the bases of the first pair of nerves is of great interest in this connection, for, as we know, the surface of the head end of the body of *Amphioxus* — especially of the young — is pigmented along certain lines, over certain areas; and it appears highly probable that the pigment cells of the surface have still some intimate relation to the light and heat perception, — one or both. The pigment of the central canal is derived from the pigment of the superficial ectoderm, which in the adult has nearly disappeared.

The primitive sense cells of the ectoderm transmitted through the nerve fibres of the first pair of nerves *general impressions*, from which those percipient elements of the brain associated with pigment cells selected vibrations of those wave lengths productive of the sensations of light and heat. The percipient elements not associated with pigment cells selected and were affected by only such stimuli as gave rise to other sensations, taste, smell, touch, etc.

Now that we have seen how most of the structural details of the brain form presented to us by all vertebrates higher than *Amphioxus* may have arisen out of the simple *Amphioxus* type, there remains for consideration a structure whose significance, both morphological and physiological, is still a matter of discussion, viz., the hypophysis. So far as I have been able to discover, there does not exist in *Amphioxus* the slightest trace of such an organ; but it is not difficult to see how such a structure could arise from the ventral portion of the thalamocœle, in connection with the further development of the mouth and its sense organs. For the present I am not in position to say more of this structure, than that the hypophysis in *Ammocoetes* is intimately related with the olfactory area, and was probably an organ of taste.

We have then, in *Amphioxus*, all the stages in the development of the paired eyes of vertebrates (as well as of the pineal or unpaired eye) demanded by the theoretical considerations, save the final ones of the formation of the completed optic vesicles and lenses. The completion of these stages occurred somewhere in the phyletic series between the *Amphioxus* condition and the Cyclostome condition. In the Cyclostomata (*Ammocoetes*) the optic vesicles grow out only slowly, and after the animal has passed the *Amphioxus* stage.

It should not be forgotten that the optic vesicle, as it pushes out from the brain, presents its anterior hemisphere to the exterior; and that in most vertebrates this half remains unpigmented, while it is this anterior portion which is pigmented in *Amphioxus*. Such must have been the primitive condition of the optic vesicle however developed.

From what has been said, it follows that there is no posterior hemisphere developed, because the process never goes far enough to form a spherical bulb, and an optic stalk.

That these differences are due to primitiveness of the organs in *Amphioxus*, and not to any inherent fundamental differences between the eyes of *Amphioxus* and the corresponding parts in other vertebrates, will be apparent to any one who will analyze and compare the earliest stages in the development of the eye in the several vertebrate groups.

We cannot believe that the paired eyes of vertebrates, as they are known from the Cyclostomes upward, sprang into existence with complete optic vesicles, optic stalks and nerves. Much less, that they were provided from the first with lens and cornea; on the contrary, the facts of ontogeny, the existence of such an idea as that of *evolution* in the domain of biological science, compel us to assume that the process was a gradual one and lasted through a long period of time, progressing only by minute increments, beginning with such a rudiment as I have shown to exist in *Amphioxus*. With the increase in complexity of organization of the type and specialization of the functions of the sense organs, came the added increments, which have resulted in the very complex, and in many ways still unknown, structure and relations of the paired and median eyes.

It is further unavoidable, that we conclude that the rudiments of eyes were functional from the very first or incipient stages through each modification up to the completed form, and that the physiological function of the organs in question have undergone as extensive, as important, and in every way a parallel growth, by slight increments acquired with the added morphological increments.

To those morphologists who object to the view I have thus briefly stated and endeavored to support, that such simple diverticula as are found in *Amphioxus* associated with pigment spots are entirely inadequate to give rise to such complicated structures as the eyes, I wish to give answer here: that we have in the case of the extremely complicated organ of the mammalian ear, an organ which has originated in a very similar manner, and whose every stage of development may be traced in existing vertebrates and about which morphologists are agreed that it is formed by simple involution of primitively superficial sense organs. The development of the cochlea, and its contained structures, is another instance of the production of an extremely complicated organ from a simple diverticulum pushed out from a

previously existing cavity, — every stage of whose phylogeny lies before us in existing vertebrates.

The chief merits of the theory I have here attempted to establish is, that no demand is made upon the organism to supply any new structures, but simply to develop organs and structures already existing, in connection with a function of which we have evidence enough that all animals endeavor to acquire in ever increasing perfection, — *i.e.* light-perception and the possibilities which grow out of it.

F. The pigment of the eye-spot of Amphioxus is contained in cells which normally lie inside the bounds of the nerve mass, and whenever found outside in microscopical preparations, it is to be considered as misplaced by chemical or mechanical means.

Sections through the *Amphioxus* brain, prepared without contraction, or tearing, from well-preserved material, never show, so far as my experience goes, the free pigment granules lying in the interspace between the anterior end of brain and its sheath. This point is worthy of mention, since the presence of such free pigment particles has given rise to erroneous ideas as to the structure of the eye-spot, and the nature of the pigment deposit.

G. The pigment bodies of the central nervous system of Amphioxus are connected with, and form a part of, segmental sensory structures.

These structures are doubtless derived from the superficial sense organs of that ancestral form in which the neural plate had not yet been converted into a tube, or had not received a protective covering.

As they exist in *Amphioxus* to-day, the regularity of their disposition is somewhat interfered with by the rearrangement of the cellular elements forming the bases of these structures, which are now transformed into a part of the central nervous apparatus. They consequently retain only indirect connection with the periphery.

After the formation of the neural plate as an embryonic organ, the cells retain their embryonic condition for a varying length of time. The transformation of these cells into the elongated and greatly enlarged spherical, functionally active

cells, occurs concomitantly with the development of the mesodermic somites and the peripheral sense organs.

This resting stage into which the cells of the medullary plate as a whole pass, as soon as the plate has been fully formed, illustrates the retardation of the development of the structure, and consequently function, of the apparatus, and this fully serves to explain why the superficial sensory organs and their pigment bodies remain undeveloped until the nervous system is entirely inclosed. For, although the medullary plate enters on a resting phase, the remaining organs of the body continue their development, especially the mesodermic structures, which by rapid growth inclose the nervous plate long before the resting phase has passed, and the differentiation of the permanent fibres and ganglion cells of the nervous cord begins.

H. Each one of the pigment bodies is connected with, forms a deposit in, an amœboid cell. All these cells retain their amœboid nature throughout life, the pigment cells of the eye-spot not excluded.

The pigment-bearing cells are relatively large, being in the majority of cases equal to the middle size ganglion cells. The pigment makes its appearance in the cells of the growing larva in the form of particles of melanin, which may or may not fuse into a single mass. The particles are related in some unknown way to the protoplasmic structure of the cell, and are controlled by the cell, massed together in a more or less irregular lump in the contracted condition, or spread out in the form of threads, sheets, rows of particles, etc., in the expanded condition, of the cell.

The pigment cells arise bilaterally near the centre of the somites, and multiply in such a way that they take in more or less of the segment longitudinally. In the contracted condition the pigment frequently appears in the form of crescentic bodies, hemispherical cups, either simple or with rays streaming out from the periphery.

The nucleus of the cell is frequently visible, though the cell wall is usually hidden by the pigment.

The nucleus is eccentric in position and in carmine stains, it colors, with its cell, much like the ganglion cells with which it is associated.

According to Stieda, the general shape of the cells during life is that of an irregular star.

It is not at all difficult to find, in the preserved *Amphioxus*, cells with several pigment processes, and often the length of the processes exceeds twice the diameter of the central mass.

I. *The pigment of the axial nervous system of Amphioxus is in process of migration towards the anterior end of the body — towards the eye.*

Where the eye is large, the interspace between it and the first pigment group is long, and *vice versa*. With the increasing opacity of the body walls, of the sheaths, and of the central nervous system itself, correlated with an increase in the degree of complexity and heightening of the function of the light-perceiving organs, the pigment cells scattered throughout the entire length of the neural axis migrate to the eye, and become associated with the percipient layer.

The interspace between the eye-spot and the first group of pigment bodies is dependent upon the size of the eye-spot — *i.e.* upon the sufficiency of its function for the body.

Stieda describes the pigment bodies as lying mostly in a plane passing horizontally through the ventral wall of the central canal; I find however that the cells oftentimes make their way dorsad along the walls of the canal, and may send processes outward from the canal reaching quite to the periphery of the cord.

J. *Rhode's conclusion that the giant ganglion cells of the anterior portion of the spinal cord of Amphioxus send out axis cylinders only caudad is erroneous, and as my preparations show, Stieda's observations are correct both in figure and text.*

Notwithstanding Rhode's positive assertion to the contrary, I am fully convinced that the giant ganglion cells show connection by means of a plurality of axis-cylinders, with structures lying both caudad and cephalad of them.

K. *The vertebrate ear has developed within the phylum above Amphioxus, and arose from one of the primary sense organs of the lateral line system, at a period phylogenetically later than the formation of the canal system of these sense organs. The ear*

organ has retained in its inclosed state the tendencies of growth possessed by the surface organs, and in the vertebrates above the Ichthyopsida offers the only remnant of the perfected primitive canal system of sense organs.

The position of the ear capsule does not mark a divide between two morphologically different portions of the brain, nor has this capsule played any part in the formation of the brain contours.

No explanation has ever been offered of the origin of those peculiar structures, the semicircular canals, of which Foster says, "But the peculiar features of the semicircular canals suggest almost irresistibly that they are special agents" in the equilibration of the body.

The solution here offered makes clear why these canals are *semicircular*, and why they, as canals, have such *special* and *morphologically significant* relations to the *sense organs* in the ampullæ, and it further helps us materially in forming a judgment as to the function of these organs which have so long proved a fruitful source of speculation, experiment, and difference of opinion.

Our knowledge of the development of the sense organs of the lateral line, and of the canal system, so intimately associated with them, we owe to several writers, but E. P. Allis has given us the most detailed and accurate account of these organs as they occur in *Amia*. Briefly stated, the steps of the developmental process are as follows: 1. The layers of the ectoderm thicken over certain areas and sink below the general level of the surface of the body. These thickened bodies lie thus in the bottom of relatively extensive surface depressions of the general body surface. The ultimate sense organs are meanwhile forming, and as they do so each sinks below the bottom of its depression and lies in a pit, the lips of which soon grow upwards and inwards towards each other. By their coalescence they form an arch over the organ, which is soon converted into a canal, by the rapid growth of the edges of the arch away from the organ, and their fusion along the median line of the depression over which they arch as they grow.

Canals originating thus, continue to grow until they meet some other canal opening, or until the exhaustion of the impulse. In the former case the two terminal pores of the canal fuse into one; in the latter the primitive canal possesses a strictly terminal

opening. The sense organs, in the meantime, have become differentiated out of the indifferent epithelium, and present the appearance of a rounded elevation covered with hair or rod-bearing sensory, columnar, epithelial cells.

The key to the solution of the whole problem lies in explaining the development of the internal ear on the basis of the development of the canal organs of the surface of the body.

When we examine the development of the canals and cochlea from the primary auditory vesicle, in the light of Allis' investigations, we find that the whole process consists in the further development within the head of one of the depressed areas and its sense organs, that are found in numbers on the body (head region) of all embryo vertebrates, and just as the primary sense organs of the surface of the body may (and usually do) produce several or many organs, so does the organ inclosed within the vesicular involution of the ear continue to multiply, until it has produced the constituent parts of the adult ear of a given vertebrate form.

Some of the deductions from these premises are as follows:—

a. Each natural group of vertebrates at the present day has a type of internal ear closely adhered to by all its members.

The so-called single semicircular canal of *Myxine* being, in reality, a double structure possessing two ampullæ and ampullar sense organs, the homology of parts between *Myxine* and *Petromyzon* is essentially complete.

b. The semicircular canals, among the *Elsamobranchs*, many times show a condition of canal development, indicative of the fusion of the primary pores at the two ends of the same canal, with an incipient separation of the primary pore thus formed from the rest of the ear.

c. In any study of the functions of the internal ear, we cannot lose sight of primitive function of the sense organ giving rise to sensory apparatus of the ear. For the functions have not suffered greater alterations than the structures, and there is no difficulty whatever in making out the structural relations of the primary and finished organs.

d. If the function of equilibration, as supposed, belongs in large part, or entirely, to the semicircular canals and their ampullar organs on account of their spatial relations, we cannot overlook

the fact that, among the lower vertebrate forms, canal organs are placed in much more favorable position (on the surface of the body) for the reception of stimuli than are the deeply buried ear canals; besides which, the former lie further from the centre of motion, and would consequently execute greater excursions than the corresponding ear canals during any given motion of the body.

These surface canals occupy positions in the three planes of space just as the ear canals do.

e. Since from an extended consideration of the experimental evidence, derived from investigations of the function of brain and ear, with special reference to the function of equilibration, we find that the semicircular canals, and their organs, are not more directly connected with the centre of equilibration than many other sense organs, and other parts of the body not sensory in function, and since we know, from their history of development, that they were purely protective structures *originally*, and have only had the semicircular form and spatial relations impressed upon them, as it seems, by the purely mechanical circumstances of involution, independently of any special function more than they possessed on the surface of the body, we must conclude *that the semicircular canals have never, and do not now, possess any peculiar or special relation to the function of equilibration.*

f. The system of canal organs is a very ancient, and, so far as the existing vertebrates are concerned, a very primitive, system. *Amphioxus* apparently has no trace of it, but all other vertebrates show, by the possession of an internal ear with semicircular canals, etc., that their ancestors must have possessed a well-developed system of canal organs in the head region.

From this primitive condition the existing variety of forms of canal organs and other sensory structures of this class have been derived.

L. *The higher sense organs of the Cyclostomata are all paired, since the nose (i.e. the nasal or olfactive epithelium) exists in the embryo as well as the adult in the form of two circumscribed areas lying on either side of the median line, each of which receives the entire nerve supply afforded by the olfactory nerve of its side.*

It is evidently not consistent with our morphological ideas, to

consider an organ unpaired and median when its *essential* structures are distinctly paired and bilaterally symmetrical, — even though some accessory portion has been so modified as to have lost all trace of its double origin. So far as I have discovered, the general acceptation has been that the Cyclostomes possessed only a single nasal organ, a single median olfactive area to which both of the olfactory nerves gave up their fibres. It is true, this supposed relation of two olfactory nerves to a single olfactive area has caused comment, and the explanation has been offered that we had to do with a modified condition, — a degradation of high sense organs. But as my sections show, the proximal portion of the nasal pit is divided by a median, non-olfactive raphe, into two lateral pockets, or *right and left nasal pits*, to which alone the olfactory nerve of the right and left sides, respectively, are distributed. The median territory is supplied by branches from another nerve, which one I have not been able to determine specifically.

I wish to call attention to the fact that, among the lower vertebrates there is a connection of the two olfactory pits across the median line by way of the mouth, here especially distinct, but among the higher forms it is also evident. This connection is usually brought about by means of grooves, placing the olfactive pit in communication with the mouth. Even in the mammalia the formation of a median partition to the extent of the formation of two *nasal canals* is a secondary process. The *primitive* or embryonic condition shows two simple pits, separated by a more or less distinct partition, which is usually a broad one. Of course in this stage the pits are not deep, and by the time their boundary walls become extensive the median portion is also well developed. In *Petromyzon* the partition remains rudimentary.

I have, from my studies on *Ammocortes* and *Petromyzon*, ventured to make my statement a general one for the Cyclostomata; and I feel sure there is no reason to doubt that *Myxine* and *Bdellostoma* will be found to have their nasal apparatus, bilaterally symmetrical, with as great distinctness as *Petromyzon* shows this very important condition. The embryonic rudiment of the nasal pits in the earliest stages of epithelial differentiation, needs further study, as none of my stages are young enough to prove that the olfactive areas arise entirely independ-

ently of each other, though I have no hesitation in venturing the prediction that this will be found to be the case.

M. *The parietal-pineal eye of the Cyclostomata and other vertebrates has been developed from a median portion of the pigmented eye of Amphioxus. The rudiments of this eye were derived from (segmental) sense organs, but the eye itself is never developed from two right and left halves, in so far as the closure of the medullary folds would necessitate this.*

No absolute demonstration of this view is at present possible, but I wish to offer the following considerations in support of it.

1. The parietal eye of vertebrates is formed as a hollow outgrowth of the anterior portion of the roof of the primitive thalamocœle, and its cells contain pigment.

2. The pigment contained within the cells of the bulbar, or functional portion of the pineal eye, is derived phylogenetically from the median portion of the pigment body of the anterior end of the primitive thalamocœle of an Amphioxus-like form.

3. The genetic connection of these three organs, for light-perception and sight, viz., the two lateral eyes and the median unpaired eye, is furthermore rendered probable by the central connection of all three organs, which is found to be in the optic thalami.

4. The same essential elements are present in each, — pigment cells and percipient rods and cones.

5. All three organs were formed to supply the demand for the restoration of the more perfect conditions for light-perception, destroyed by the folding in of the medullary canal.

6. The two kinds of eyes were primarily alike in structure and lensless; both formed lenses, the paired eyes first, — and this condition has been retained by all descendants, the pineal eye only in certain forms, — ancestors of the groups still showing traces of the lens body, and their descendants. The double nature of this organ, recently described in Leydig and Selenka, does not affect the above views, for, so far as we yet know (and the matter needs further investigation), both of the tubular outgrowths, although intimately related, are materially different in one respect, viz., only *one*, the epiphysis, contains pigment in its distal portion.

N. *The neural axis of all vertebrates is co-extensive with that of the chorda, or vice versa, since the neural axis is phylogenetically as well as ontogenetically the older structure.*

In this I can confirm Keibel's observation on mammals, by my own on Sharks. The same is true of the early larval Cyclostomata and Amphioxus. My own results, the outcome of a study of *Acanthias vulgaris* and *Galeus canis*, at the Maine Biological Laboratory, Wood's Holl, Mass., were obtained several months before the publication of Dr. Keibel's paper. This relation of chorda and neural axis exists, not only in all the higher vertebrates, but in *Amphioxus* as well, during the stage in which the organs in question arrive at their complete separation from the surrounding tissues; but while in higher vertebrates the notochord suffers a shortening of its anterior end, and not infrequently of its posterior end also, in *Amphioxus* the anterior end secondarily grows out into a process for the support of the pointed anterior end of the head.

A cephalic flexure occurs in both, and is more or less completely obliterated in both in the later stages, though from different causes.

In no craniate vertebrate does the notochord extend the entire length of the embryo when first formed, but ends in an undifferentiated mass of cells placed below and in front of the neural axis, and later grows out in front of it. This outgrowth is soon overcome by the much greater and more rapid growth of the brain. The conditions in *Amphioxus* are simpler in that the notochord is, from the first, distinct quite to the end of the neural axis, the anterior termination of the body being relatively much larger than in embryo of the higher vertebrata of corresponding stages.

O. *The pituitary prominence of the skulls of vertebrates does not mark a fixed point, as the relation of the anterior end of the chorda and of the hypophyseal organs clearly proves.*

I reserve a detailed consideration of this proposition for my illustrated publication.

P. *In the discussion of the segmentation of the head it has become necessary to deny any segmental value whatsoever to any portion of the chondro, — or ossicranium. They have no greater segmental value than the intestine. And all apparent segmental*

characters have been impressed upon them by other organs of segmental nature. Not only does ontology force this conclusion, but the historical development of our anatomical knowledge alike compels its admission.

It is taken for granted that the head region has been formed by a gradual process of modification of the anterior end of some ancestral type, whose body, with the exception of the first and last segment, was composed of nearly, if not quite, homodynamous segments. The problems are to determine what was the value of these primitive segments, and how they have been so modified as to produce the head. Obviously the only way open to the morphologist is to determine what existing vertebrates show the steps of this process, and the extent to which the primitive segmentation persists in the highly differentiated vertebrates of this age.

Few if any topics of vertebrate anatomy have received the attention of so many morphologists, or have been discussed with such interest, as the nature and development of the head.

It has long been recognized that within the vertebrate phylum the cephalon undergoes a most wonderful degree of differentiation, unequalled by any other portion of the body. As the lowest step in this series we have *Amphioxus* with the head less developed, both morphologically and physiologically, than many annelids and arthropods, — with this reservation of course, that in the Lancelet, as we say, the type is higher. From *Amphioxus* to the next stage, as represented by the Cyclostomes, the transitional forms are unknown, but the larval development of *Petromyzon* gives some interesting hints as to the manner in which these transitional stages have been passed through. From the Cyclostomes up to man, the series is practically unbroken, and allows us to trace, with a great degree of accuracy, the course of development, and to formulate some ideas as to the causes which have been at work effecting the progress.

Morphological conclusions, in the realm of neurology, have undergone great modification within the last decade, and as our knowledge increases we have found it necessary to widen our views as to what is normal and essential for a neural segment. From a general survey of the field, it is clear that a classification of the cephalic structures of modern vertebrates must be made, separating those which are genetically connected

with the primitive organs, persisting with no change of function from those which have appeared much later and in a modified condition of the body. The brain is of the former class, the cranium of the latter. As an illustration, we may take the cases found in nature of a completely segmented vertebral column and its separation from the skull by an articulation, and the opposite condition of a continuous cartilaginous skeleton, including vertebral column and cranium. The explanation current is, that in the latter case we have to do with secondary fusion, and that we have here a modified condition derived from the typically segmented condition by a lack of development of the articulation. I consider it the true view, that a continuous cartilaginous skeleton has been, and is in some living cases, the primitive and normal condition, though of course I do not deny the production of more or less continuous portions of the skeleton by a process of secondary fusion.

The segmentation of the contained or enveloping organs does not predicate the segmentation of the containing or enveloping structure, any more than the formation of an ear capsule of a mammal, out of separate bones, predicates the segmentation of the sense organ contained.

Q. The head cavities, or spaces included within the mesoblastic somites occurring in the head region, possess relatively the greatest importance in an acraniate stage before a skull or anything comparable with a primordial cranium has made its appearance. This is true from the ontogenetic as well as phylogenetic standpoint.

R. The hypophysis is a structure which arose in the vertebrate phylum long after the chorda was established, as Amphioxus proves, and was connected in an important way with the infundibulum. It arose as an organ of taste, and the infundibulum was its nerve.

S. The optic chiasm (the trochlear chiasm as well) has arisen within the vertebrate group above the Amphioxus condition and in the following manner:—

The fibres supplying the pigment spots (or muscle) arose from ganglion cells of the multipolar kind, and as we know in Amphioxus and Cyclostomes, these fibres cross the middle line not

unfrequently, or the cells lie in the axial line of the nerve cord or brain. By a gradual process of development, it came about that the majority of fibres for the right side of the body found their central ends in the left side of the brain, for some as yet unknown cause, although this transposition was probably intimately connected with the development of the system of association fibres.

T. *The explanation of the increased number of gill slits of Amphioxus over those of other vertebrates (which certainly show traces of considerable reduction in number) is to be found in the habits of the Amphioxus, which is not a free swimming animal, and cannot be a predatory one. It depends, for its food, upon the size and power of its branchial apparatus to create currents and keep moving a sufficient volume of water to supply it with the requisite amount of food, which is contained in only limited quantities therein.*

As the animal grows larger its needs are greater; the branchial chamber and apparatus must increase to allow a larger volume of water to pass.

U. *The branchial apparatus of Amphioxus is then not merely a respiratory apparatus, but more an apparatus for the collection of food and for the transfer of such collected store to the pharyngeal opening for deglutition. A much smaller organ than the branchial basket of the adult animal would suffice for the adequate respiration.*

We have only to call to mind the peculiarly inactive life that the animal leads, to become satisfied that its so-called respiratory apparatus is much more important as a food collector and strainer than as a respiration organ.

This modification of the branchial apparatus, for food collection, is paralleled in higher vertebrate by the production of mandibulo-maxillary region for the same purpose.

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STUDIES ON CEPHALOPODS.

I.

CLEAVAGE OF THE OVUM.

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I.

IN the following pages, I will first attempt to treat the general morphology of the animal ovum from the standpoint of some embryological and morphological facts and theories. In the next place, the relation of the external phenomena of cleavage, as shown by the behavior of the cytoplasm, to the internal phenomena of nuclear cleavage or caryokinesis will be discussed. In this connection, some theories on caryokinesis will be examined, my interpretation of the cleavage phenomena being that they are essentially the *analysis* of the potential tissues contained in the cleavage nucleus, and that caryokinesis

is the *method* of such analysis, and the achromatic spindle the *instrument* used in the analysis. The cleavage of the squid ovum will then be described, and finally variations in the cleavage of the same animal will be discussed.

The observations were made during the summers of 1888 and 1889, while I was a student at the Johns Hopkins University, at the Woods Holl Laboratory of the U. S. Fish Commission, through the kindness of Professor M. MacDonald.

The pressure of other studies at the time and the difficulty of obtaining a constant supply of the most desirable stages have left my observations incomplete in many respects. Although the squid eggs are abundant during the summer months at Woods Holl, it is difficult to get the early stages, since the bunches of egg-capsules caught in a dredge or in a trawl are usually too far advanced for the study of cleavage. The following observations were made on material obtained from two female squids. On the night of September 3, 1888, one squid, *Loligo Pealei*, kept in an aquarium, laid four capsules of eggs. The eggs were examined immediately, and the cleavage stages were followed. All the figures on Pls. IX and XII, except Fig. 29, were drawn from specimens obtained from this source. Subsequently I killed the same squid and fertilized the remaining ova artificially. A few better drawings of the surface views of the dividing eggs were thus obtained.

One day in August, 1889, another specimen of the female squid with ripe eggs was kindly given to me by Professor F. M. McFarland of the Marine Biological Laboratory. The eggs were taken out and artificially fertilized with spermatozoa which were found in the spermatophores in the inside of the external buccal membrane of the same animal. All the figures in Pls. X and XI, and Fig. 29 in Pl. XII, were drawn from this material. Judging from my experience, I believe that the study of the early stages of cleavage is best accomplished with artificially fertilized ova. Such ova have many advantages over those laid naturally. In the first place, the eggs can be studied free from the jelly covering, which interferes in no small measure with the process of successful manipulation. In the second place, the ova may be obtained at any stage of their cleavage at the time when it is most convenient to work.

If one succeed in catching a gravid female, it is an easy mat-

ter to get hundreds of ripe ova from the egg-reservoir. The eggs, when ripe, shine through the thin wall of the body underneath the mantle, and easily roll out through an incision in the wall, as the shot would do through a similar opening in the sac which contained them. A search for the spermatophores may then be made in the adult male, which, in the reproductive season, carries several bunches in the inside of the mantle chamber. If the search in the mantle chamber fail, we can usually get quantities of them in the reservoir, through the thin walls of which can be seen, regularly arranged, white, elongated, spindle-shaped spermatophores. Search for a male is often rendered unnecessary, for a number of spermatophores are usually carried by the females on the inside of the arms, and may usually be found on the little horseshoe-shaped prominence on the inner surface of the outer buccal membrane of the same animal from which the eggs were taken. This is a matter of great convenience to the investigator, for a single female with well-matured eggs will usually have spermatozoa enough to fertilize all the eggs. Taking hold of the bunch of spermatophores with the forceps and shaking them into a dish with a little water in it, the spermatozoa can be liberated easily. The process can be facilitated by cutting the spermatophores with scissors into a few fragments. By pouring the water, fragments of the spermatophores and all, over the eggs, and mixing them thoroughly, the artificial part of the fertilization may be said to have been accomplished. If one take an egg and a few drops of the water after this process and examine them under the microscope, one will see a multitude of spermatozoa covering the surface of the ovum, and with little perseverance one can follow the penetration of the sperm-cell into the ovum through the micropyle. Between one and two hours after the mixture of the sperm and the ova, the first furrow of the cleavage was observed, and thence at intervals of five to ten minutes, the succeeding furrows of cleavage were introduced, the interval between the two successive furrows becoming shorter and shorter as the stage advances.

After the majority of ova have commenced to divide, it is necessary to change the water frequently, particularly when a large number of eggs are kept in the same vessel.

In order to separate the blastoderm, the egg at any given stage of division was killed by a mixture of sea-water, acetic

acid, and osmic acid, in the proportion recommended by the Hertwigs for their well-known macerating fluid. The proportion of the osmic acid may be reduced, or may be altogether excluded from the reagent, as it affects the eyes of the observer while he is closely watching the effect of the reagent upon the egg and teasing away the blastoderm with needles. I found it most satisfactory to remove the ovum from the action of the reagent as soon as the translucent protoplasmic germ-disc turns whitish opaque, which takes place very quickly, and complete the operation of separating the blastoderm in dilute glycerine.

The blastoderm thus separated may afterwards be stained with dilute Schneider's acetic carmine, or with Erlich's hæmatoxylin, and then mounted in glycerine; or may be left unstained, as most things can be satisfactorily made out without any staining, and by avoiding this unnecessary process of technique in this case, the risk of dislocating or injuring the delicate blastoderm in other ways is thus greatly lessened — the risk all the more to be avoided as the blastoderm is not firmly fixed to the slide, and a little movement of the cover-glass is often enough to destroy the whole specimen.

Treatment of living eggs with Perenyi's fluid for a few seconds is excellent for the surface study of cleavage. It turns the protoplasmic portion of the ovum opaque yellow, and brings out the cleavage furrows distinctly, while the rest of the egg remains translucent as before.

II.

It was a disputed question at the time when the cell-theory was first promulgated, how much of the animal ovum is to be homologized with Schwann's scheme of an organized cell — whether the germinal vesicle alone is to be taken as such, or the whole substance of the ovum, including both the cytoplasm and the germinal vesicle.

This failure to recognize the true cellular nature of the ovum and of the cleavage segments, coupled with the false doctrine of the Cytoblastema of Schwann, stood in the way of a true understanding of the cleavage process, and obscured the significance of the cell-theory itself.

Our understanding of the cell-theory was made possible (1) by

the accurate determination of the ovum as a single cell; (2) and by the identification of cleavage with cell-division.

In regard to the first question, although Schwann, after considerable hesitation, inclined to the conclusion that the ovum is morphologically a cell, and that the germinal vesicle is its nucleus, still he felt compelled by the evidences available in his time to admit that "it was impossible to decide the question whether the germinal vesicle be cell or nucleus."

In regard to the second question, he was totally unaware of its true significance, as his theory of the Cytoblastema very well shows. Within a few years of the publication of Schwann's great work, however, it was definitely determined that the cleavage of the ovum is a process of cell-multiplication; and that in the whole domain of developmental phenomena of animal tissues no cell-formation ever takes place independently of those cells already existing in the organism.

Following the identification of the germinal vesicle of the ovum as the true homologue of a typical cell-nucleus, and the cleavage of the ovum as a process of cell-formation, came the problem of the promorphological character of the ovum. This is an entirely distinct problem from that of the germ-layers, and is essentially the outcome of a more careful investigation into the early phenomena of egg-development. The existence of the germ-layer theory did not depend on morphological views of the ovum. The germ-layers were recognized and studied before naturalists knew anything about cleavage and the morphology of the ovum. The cytological method deals with facts before the cells arrange themselves into definite layers. It deals with a certain cell or a certain group of cells in an early stage of cleavage, from which different embryonic organs and germ-layers themselves are derived. For example, it traces the mesoderm to a pair of cells, or the future ectoderm and endoderm to the two-cell stage of development. It seeks to explain the differentiation of the axes of the adult or larval organism by tracing them to certain recognizable axes in the extremely early stages of development, or even in the unsegmented ovum itself.

It seeks to find out the significance of the different planes of cleavage in their relation to the origin of tissues in the larva or the adult. Thus, instead of starting with the formation of the germ-layers in the study of organogeny, it goes a step further

and attempts to derive those germ-layers themselves from a simpler cleavage segment or segments; and instead of comparing well-arranged layers in the establishment of homologies of structures in different organisms, it seeks to reduce them to a still simpler aggregation of cells. The teloblasts of Whitman and Wilson, in which an early differentiation of the tissue-germs is recognized, furnish admirable examples of this class of phenomena. May not the well-known fact of the early separation of sexual cells in the body of a metazoan embryo belong to the same category of facts?

While the teloblasts represent only a part of the entire organism, as, for example, the nervous system or the excretory organ, the sexual cells contain the "Anlage" of the complete organism. The difference appears to me to be more one of degree than of kind. While the one completes its development during the ontogeny of the organism, the other completes its development at the next generation.

Although this tendency to recognize early differentiation of parts in an early stage of the dividing ovum is essentially the outcome of more recent studies, it was foreshadowed in the works of several earlier investigators. Von Baer, as far back as 1834, recognized the animal and vegetable poles in the frog's ovum. Newport, as far back as 1854, identified by an ingenious device the first cleavage plane of the ovum with the median axis of the embryo in the frog, and pointed out the origin of the head and tail end of the embryo from definite parts of the ovum. Pflüger and Roux have more recently shown the coincidence of the median axis of the embryo with the plane of the first cleavage furrow in the ovum of the frog. The striking series of results brought out by the latter in experimental embryology are the most important contributions in this connection. Mark has discussed the "primitive axis" of the ovum in *Limax*; Goette has fully treated the relation of the promorphological axes of the ovum in the annelid, and their relation to the cleavage and early differentiation of tissue-cells; Hatschek has pointed out the early exhibition of the bilaterality in the ova of *Teredo* and *Pedicellina*; Brooks, in the ova of the American oyster. E. van Beneden, in various papers on *Ascaris*, *Tunicates*, etc., has called particular attention to the importance of the study of the promorphological relation of the ovum.

Agassiz and Whitman have most fully discussed the importance of this question, while Hallez was led to the formulation of an important law in regard to the orientation of insect ova. Blochmann and Wheeler have described the early exhibition of the bilateral arrangement in insect ova, extending even to the distribution of yolk-granules.

Several others may be mentioned whose work bears more or less on this important problem. Some of these we will examine more in detail later. It may be pointed out, however, at present that in the study of segmentation of the ovum, a mere quantitative enumeration of blastomeres at the successive stages of egg-cleavage alone, has ceased to be a matter of any great importance.

Van Beneden and Julin¹ have demonstrated in the egg of *Clavellina* the existence of a bilateral axis before it begins to segment. The anterior and posterior extremities, the left and right sides, the dorsal and ventral aspects of the larva can be definitely pointed out in the unsegmented ovum. The first furrow of cleavage coincides with the median plane of the bilateral larva, the left half of the larval organization being derived from the first primary blastomere which lies on the left side of the first furrow of cleavage, and the right half of the body, from the right cleavage sphere. Properly speaking, the body of the larval *Clavellina* consists of two identical halves which meet in the median line. All structures in the body are paired, or have double origin corresponding to the bilateral symmetry of the ovum. The median structures, such as the notochord, digestive tube, are derived from two sources,—the right and left halves of the body,—although later, one side of the body may be invaded by the cells from the other side.

Chabry,² who worked on *Ascidia aspersa*, has confirmed the results of the above-mentioned authors, and has established with certainty the primitively double character of such median structures as the eye, otolith, notochord, the atrium, and the organ of fixation. He thinks that the eye in the adult is derived from the cells situated in the anterior end of the right half of the body of the larva.

¹ Ed. van Beneden et C. Julin: *La segmentation chez les Ascidiens et ses rapports avec l'organisation de la larve*. Archives de Biologie, Tome V, 1884.

² *La segmentation chez les Ascidiées simples*. Jour. Anat. et Physiol., T. 20, 1885.

Van Beneden¹ in his recent work with Neyt, on *Ascaris*, is inclined to carry his former conclusion still further, and believes that the ovum as well as the other cells of the body probably have the inherent bilateral structure, and traces the bilateral symmetry of all Metazoa, either in the larval stages or retained through their life histories, to the bilateral structure of the unsegmented ova themselves. "Les premiers blastomères ont, comme l'œuf fécondé, non seulement une symétrie mono-axone, mais une structure bilatérale. Il est probable que c'est là un caractère comme à toute cellule et l'on doit concevoir un organisme cellulaire, non comme formé de couches concentriques, mais comme présentant essentiellement un axe à extrémités différentes et un plan unique de symétrie. Cette symétrie bilatéral de la cellule est probablement la cause de la symétrie bilaterale des organismes plus complexes, des animaux en particulier. Il est bien prouvé maintenant que la symétrie bilaterale est primordiale chez les Radiaires, les Echinodermes, et les Zoophytes, comme chez les Mollusques, les Vers, les Arthropodes et les Chordés : la symétrie radiée n'apparaît que secondairment chez les Echinodermes et les Coelentérés. Nous pensons que la même démonstration sera faite un jour pour les protozoaires et pour les végétaux."

Hallez,² who paid especial attention to the promorphology of the ova of insects, finds that there is a definite law of orientation in the ova, a law which he restricts to insects at present. The law runs as follows : "*La cellule-œuf possède la même orientation que l'organisme maternel qui l'a produite : elle a un pôle cephalique et un pôle caudal, un côté droite et un côté gauche, une face dorsal et une face ventral ; et ces différentes faces de la cellule-œuf coïncident aux faces correspondantes de l'embryon.*" Hallez observes elsewhere³ that, "L'œuf, pendant une période de son histoire, faite partie de l'organisme maternelle à titre de simple élément histologique. Or, les expériences de sections et de régénérations, faites sur les Planaires et autres animaux, montrent que chaque tronçon, si petit qu'il soit, conserve la même orientation, c'est-à-dire les deux polarités céphalique et caudal,

¹ *Nouvelles recherches sur la fécondation et la division mitotique chez l'Ascaris mégalocéphale.* 1887.

² Hallez : *Comptes Rendus*, 103, 1886.

³ Hallez : *Comptes Rendus*, 101, 1885.

qu'il avait dans l'animal entier. C'est quelque chose de comparable à l'expérience de l'aimant brisé. Ne peut-on en conclure que chaque élément histologique possède, lui aussi, ces deux polarités de l'animal, polarités qui persisteraient dans la cellule-œuf, après qu'elle a cessé de faire partie des tissus maternels?"

Mark¹ has recently called attention to the fact that in the *ovarian ovum* of *Lepidosteus*, the polar differentiation of the ovum can already be pointed out by the existence of a peculiar secretory activity of the egg-protoplasm at one point, which corresponds to the future micropyle region. According to Mark, the egg-membranes of the ovum — the villous layer and the zona radiata — are produced by the secretion from the surface of the ovum, and the change in the egg-membranes corresponding to the region of the future micropyle is due to the diminished secretory activity of that particular part of the ovum.

Mention must be made in this connection of the important fact established by Rabl,² Carnoy,³ and Gehuchten,⁴ that the resting nuclei in the tissue-cells of certain animals, such as the epithelial cells of the Salamander, the testis cells of a certain Arachnid and of Crustacea, the intestinal cells of a larval Dipteron, there are either two poles or an axis, around which chromosomes arrange themselves in a definite way.

The preceding, taken somewhat at random, will show one essential fact; viz. that, however diverse the examples, they all point to one and the same conclusion, namely, that the metazoan ovum and its derivatives, the tissue-cells, are more than a homogeneous, isotropic mass of protoplasm, devoid of definite symmetry. The study of caryokinetic figure shows, van Beneden points out, that the cell is not only uniaxial, but also bilateral. In several forms of ova carefully studied, the axes of the caryokinetic figure correspond in a definite way with the recognizable axes of a given ovum, the external shape of which is chiefly determined by the quantity and distribution of the food-yolk. The axes thus determined are maintained through the differ-

¹ E. L. Mark: *Studies on Lepidosteus*. Bull. Mus. Comp. Zool. Harvard College. Vol. XIX, No. 1, 1890.

² Rabl: *Ueber Zelltheilung*, Morph. Jahrb., Bd. X, 1885; *Ueber Zelltheilung*, Anat. Anzeiger, IV, Jahrg. No. 1, 1889.

³ Carnoy: *La Cytodiérèse chez la Arthropodes*. La Cellule, Tome I, 1885.

⁴ Gehuchten: *L'Axe organique du noyau*. La Cellule, Tome V, 1889.

ent stages of growth and give rise to definite axes of the larval or of the adult organism. If these facts be more firmly established by the further investigation of the subject, we may say with van Beneden:¹ "L'ancienne théorie de l'évolution ne serait pas aussi dénuée de tout fondement qu'on le croit aujourd'hui."

III.

It has been pointed out that the cytological study of the animal ovum is different from the study of germ-layers. It has also been pointed out that the morphology of a bilateral organism, at least in those well-established cases, has its beginning in the morphology of its bilateral ovum.

The next important point to be borne in mind, it appears to me, in the study of the cleavage of the ovum, is the phenomena of tissue-differentiation, which become more and more manifest with the growth of the ovum and the progress of its cleavage. Now what is meant by the differentiation of tissues? What is the relation of the tissues thus differentiated to the original ovum from which they sprung? How does one tissue-cell differ from another, which belongs to another tissue?

Strictly speaking, the differentiation of tissues in the developing organism means the formation of two or more different kinds of protoplasm out of one. For the sake of clearness we may begin our inquiry by asking, How does one egg which is a single nucleated cell, and which gives rise to one animal, differ from another which gives rise to an entirely different organism? Both are simple nucleated cells, and as such they are morphologically alike, but one egg will develop into one organism and the other into another after a repeated division and subdivision, exactly under the same circumstances, and often in the same space of time. The difference in result cannot, therefore, be attributed to difference of conditions under which they develop, but to something inherent in the ova themselves. In other words, the egg-cell of a jelly-fish must have had from the beginning the potentiality of becoming a jelly-fish and nothing else; and similarly, the starfish ovum must have been a potential starfish from the beginning. To imagine, therefore, that all proto-

¹ Van Beneden: *Recherches sur la maturation de l'œuf, la fécondation et la division cellulaire*. Archives de Biologie, Tome IV, 1883.

plasm is identical, because no difference is recognizable by any means at our disposal, would be an error. Deep within the two particles of protoplasm which give rise to two different organisms, there must be a corresponding difference which lies at the bottom of all differences. In short, the eggs of two different animals must be supposed to differ in their elementary constitution, as much as their adult organisms differ in anatomical structure. "From general scientific principles," says Professor Sachs,¹ "we must assume *that for each visible external difference of organ, there is a corresponding difference in its material substance*, exactly as we regard the form of a crystal as an expression of the material properties of the crystallizing substance." And again, says the distinguished German botanist, "Even the different shapes of the two sexual cells — of an antherozoid or a pollen grain compared with the oösphere — indicate plainly that both are constituted differently as to material, since the external form as well as the internal structure of any body is the necessary expression of its material constitution. Difference of form always indicates difference of material substance." This doctrine of *Form and Matter*, or of *Mechanism and Function* as expressed in the language of physiology, is the basis of our biological inquiries. As is clearly expressed in the words of Professor Burdon Sanderson,² we must assume "*that every appreciable difference of structure corresponds to a difference of function; and conversely, each endowment of a living organ must be explained, if explained at all, as springing from its structure*"; or, in short, we must hold to the principle "*that living material acts by virtue of its structure, provided we allow the term structure to be used in a sense which carries it beyond the limits of anatomical investigation, i.e. beyond the knowledge which can be attained either by the scalpel or the microscope*." Given protoplasm of definite structure, and we have its definite function or property. Or conversely, we observe a certain property in a given mass of protoplasm, and we regard it as springing from a definite structure. When structure varies, the function must vary also; and when we observe certain peculiar properties we must ascribe them to peculiarities in structure.

¹ Sachs: *Lectures on the Physiology of Plants*. 1887.

² Burdon Sanderson: *Presidential Address to the Section of Biology, British Association for the Advancement of Science*, 1889. *Nature*, Vol. 40, No. 22.

One rational answer to our inquiry is possible; viz. the protoplasmic structure of the egg which gives rise to one organism, must differ from that of the egg which gives rise to another different organism, the differences between the two being relatively as great as those which the two adult organisms display in their anatomical relationships.

If the similarities of two organisms must be attributed to the corresponding similarities of the protoplasmic structure of the fertilized ova from which they respectively arise, the source of similarities in the latter must be sought for in the community of their hereditary antecedents. Hence, one way to place the doctrine of phyletic kinship of two or more organisms upon a scientific basis, would be, if such a thing were possible, to demonstrate the molecular or structural affinity of their germ-cells. The embryological phenomena of a developing organism may be expressed in the terms of protoplasmic metamorphosis. Two organisms at the same stage of development would represent the same stage of protoplasmic structure. The budding of a new cell or the formation of a new organ would correspond to the birth of a new phase in the course of the metamorphosis of the original protoplasm of the egg.

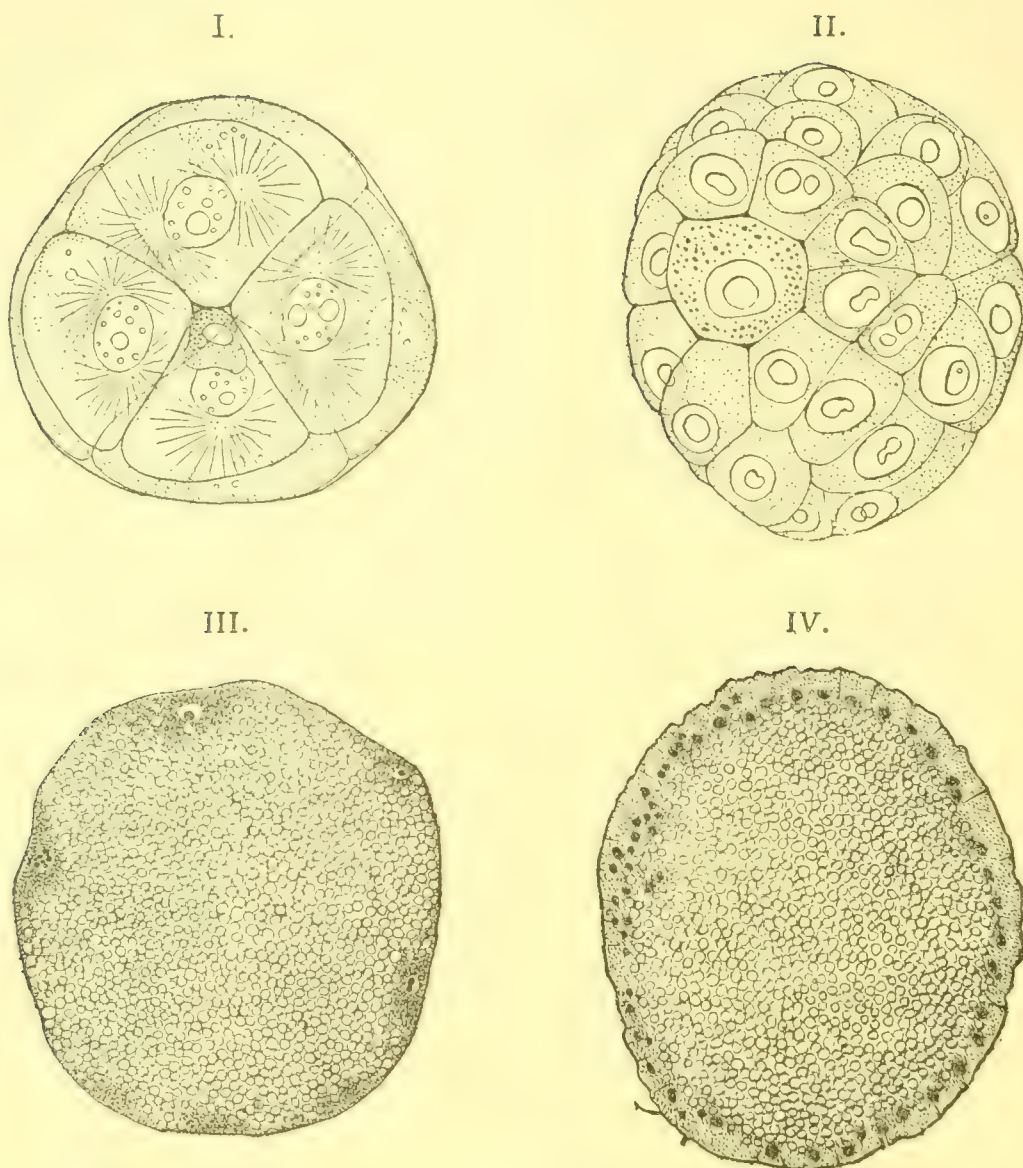
To turn to our main point, namely, the development of the organism as first indicated by the cleavage of its protoplasmic material.

What is the cleavage of the ovum? What is accomplished by it? Is it the mere sundering of material which has no more reference to the future organization of the embryo than the snowflakes bear to the size and shape of a future avalanche? Or is it a "histogenetic sundering" of material in which every step in the process has a definite relation to the building up of the future embryo? That each step of cleavage has some definite significance in relation to the organization of the adult or the larva, at least in certain forms which have been most carefully studied, there can be no question. Thus, in a certain Tunicate already referred to, it has been observed that the nuclear substance of the ovum is divided, during the first cleavage, in such a manner that one of the new nuclei, by its division, gives rise to the right, and the other to the left side of the adult organism. In another case, as in some worms, it has been maintained, that the first division of the nucleus distributes the nuclear substance

into future ectoderm and entoderm. And again, the formation of a certain organ, or a system of tissues, has been traced in a most definite manner to a particular cell or group of cells in an early stage of cleavage, as is shown by the well-known researches of Whitman on *Clepsine*, or more recently in the admirable work of Wilson on *Lumbricus* and *Nereis*. The more carefully the phenomena are studied, the more astonishing is the regularity and precision with which the cleavage process is conducted and the differentiation of tissues is accomplished.

The occurrence of variations or irregularities in the mode of cleavage in a certain animal — irregularities as judged by the arrangement of superficial cytoplasmic furrows — does not invalidate the importance of the conclusion which can be derived from the study of forms where a great regularity prevails. For the essential feature of the cleavage process is the division and distribution of the nuclear substance of the ovum, and in so far as the nuclear substance is distributed in such a manner as to produce a symmetry of growth in the developing organism, it is immaterial whether its total quantity be divided exactly in two equal halves and distributed into right and left at the first cleavage, or whether it be divided into dissimilar portions and the equilibrium of growth be gradually secured during the subsequent stages of cleavage. The distribution of the nuclear substance may have been just as accurate and precise in one case as in the other.

A comparative study of cleavage of different ova affords another example illustrating this point. For instance, as my friend Dr. C. Ishikawa tells me, the summer and winter eggs of a certain form of *Daphnidæ* undergo different "types" of cleavage, one being holoblastic and the other being meroblastic, the difference being probably produced by the amount of food-yolk. Dr. Ishikawa has kindly placed at my disposal several interesting drawings which illustrate this point clearly. The accompanying figures show the difference of the modes of cleavage between the yolkless summer egg and the winter egg, in which food-yolk is abundant, *from one and the same species of animal*. The summer egg belongs to the regular, holoblastic type of cleavage, and the winter egg, to the meroblastic type, showing a close resemblance to the ova of some insects.



Polyphemus oculus :

Figures I and II show the holoblastic cleavage of the summer eggs.

Figures III and IV show the meroblastic cleavage of the winter eggs. All from unpublished drawings of Dr. C. Ishikawa.

From Dr. Ishikawa's private communication, as well as from the published account by Grobben,¹ Weismann and Ishikawa,²

¹ Grobben: *Die Embryonalentwicklung von Moina rectirostris*. Wiener Arbeiten, Bd. II, 1879.

² Weismann and Ishikawa: *Ueber die Bildung der Richtungskörper bei thierischen Eiern*. Ber. d. naturf. Gesellsch. zu Freiburg, Bd. III, 1887, Heft 1, Taf. I, II, and III. *Ibid.*: *Ueber die Paracopulation im Daphnidenei*. Zoologische Jahrbücher, Bd. IV, 1889, Heft 1, Taf. X, XI, XII, XIII.

on the early development phenomena of the various forms of Daphnidæ, I constructed the following table, which shows at a glance the intimate connection existing between the so-called "types" of cleavage and the relative amount of deutoplasm contained in the protoplasm of the egg, and this conclusively, because the difference in cleavage is seen in the ova of one and the same species of animal.

	Broods of Eggs.	Types of Cleavage.	Quantity of food-yolk.
<i>Polyphemus oculus</i>	{ Summer	holoblastic.....	scanty.
	{ Winter	meroblastic.....	plenty.
<i>Bythotrephes longimanus</i>	{ Summer	holoblastic.....	scanty.
	{ Winter	meroblastic.....	plenty.
<i>Moina rectirostris</i>	{ Summer	holoblastic.....	scanty.
	{ Winter	meroblastic.....	plenty.
<i>Leptodora hyalina</i>	{ Summer	meroblastic.....	plenty.
	{ Winter	meroblastic.....	plenty.
<i>Daphnia longispina</i>	{ Summer	meroblastic.....	plenty.
	{ Winter	meroblastic.....	plenty.

In *Leptodora* and *Daphnia*, both the winter and summer eggs contain plenty of food-yolk, so that the cleavage is not complete; while in *Polyphemus*, *Bythotrephes*, and *Moina*, the difference between the cleavage of winter and summer eggs are so sharply marked that the two broods of eggs may be taken for two entirely different organisms, if the affinity of the "types" of cleavage of the ova were in any way indicative of the systematic affinity of the adult organisms. The same may be said in regard to the cleavages in different species of *Peripatus*, as the studies of Sedgwick and others have shown. The same is true in the case of *Renilla*, as was shown by Wilson. In short, if we classify animals by the "types" of cleavage, or differences of cleavage, rather than with reference to the potential qualities of the nuclear substance, we fall into an error of placing nearly related species of organisms in different categories; nay, we even fall into the absurdity of separating the individuals of one and the same species into different groups.

Sachs¹ gives an instance illustrating the same fact in plants; viz. six different forms of cleavage taking place in the pollen mother-cells from one and the same orchid, the difference in cleavage being produced by the slight individual variation in

¹ *loc. cit.*

the outline of the original mother-cells. He points out that the mode of cytoplasmic cleavage of cells depends chiefly upon the configuration of a growing organ, and I may add, of an organism, at the time when it begins to grow, and not upon its phyletic significance. As an example, he cites the cleavage of cells in a glandular hair of the gourd-plant and the embryo of a Phanerogam, both of which undergo essentially the same "type" of cleavage in every detail so far as we can judge from the resemblances in the external appearances of their cleavage furrows.

Balfour pointed out long ago that the similarity or dissimilarity of the cleavage process in the ova of different animals as indicated by the external phenomena alone, has no value whatever in estimating the systematic affinities of different organisms which develop from them. Given two animal ova with their external configurations alike, owing to the similar distribution of food-yolk and of the germ-protoplasm, then the early phenomena of cleavage will be also alike, whatever may be the difference of animals which later develop from them.

That the argument based on the arrangement of superficial furrows alone is not entitled to any weight, is further shown by their total absence in several forms of ova, which nevertheless develop into perfect organisms. It has been shown that in a certain plant, the cytoplasm becomes divided without a corresponding division of its nucleus.¹ Such facts seem to point to the conclusion that the division of the cytoplasm and that of the nucleus are two independent phenomena, and that one process can occur without the other, and that when they do occur in close succession, as in ordinary cell-division, it is to be looked upon as a case of coincidence.² At any rate, the following conclusion seems to be a valid one; viz. that the division of the nucleus and that of the cytoplasm are due to different causes. The cleavage furrows are devoid of significance, taken by themselves. The formation of a cleavage furrow is a negative phenomenon, a reflex manifestation of some other activities going on elsewhere.

It is now quite generally conceded that the nucleus of the

¹ For different examples in which the caryokinetic division of the nucleus is not followed by the division of the cytoplasm, see Kölliker: *Handbuch der Gewebelehre*. 6th edition, p. 61, 1889.

² Sachs: *Lectures on the Physiology of Plants*. 1887.

fertilized ovum contains all the hereditary characteristics of the parent organism. It is this structure in the ovum which stamps the particular characteristics upon an organism of a given species. The study of fertilization has clearly demonstrated the metamorphosis of the sperm-nucleus into a constituent part of the cleavage-nucleus, and thence it is distributed to all nuclei formed in the subsequent cleavages. Morphologically, all the hereditary characteristics which the infant organisms inherit from the parents, must be traced back to a certain number of chromosomes which come from the sperm and egg-nuclei of the fertilized ovum. By cleavage, the potential characteristics become gradually analyzed into their special attributes — the attributes which we assign to different tissues of the larval or the adult organism. If, therefore, I may use one word to characterize the whole process of cleavage of the ovum, the term *Analysis* will perhaps best express our interpretation of the phenomenon. It is true that we know very little as to the essential respects in which the nuclear substance in the entodermic cleavage sphere differs from the similar substance in the ectodermic sphere. In the present state of our knowledge on this subject, we can only infer a structural difference of the protoplasm from the careful study of the fate of the respective segments. If, for instance, one cell gives rise to a sense-organ, the fundamental molecular structure of that cell must be different from another which contains all the germs of an excretory organ, just as we are forced to conclude that the ova of different organisms are of necessity different, even if they appear

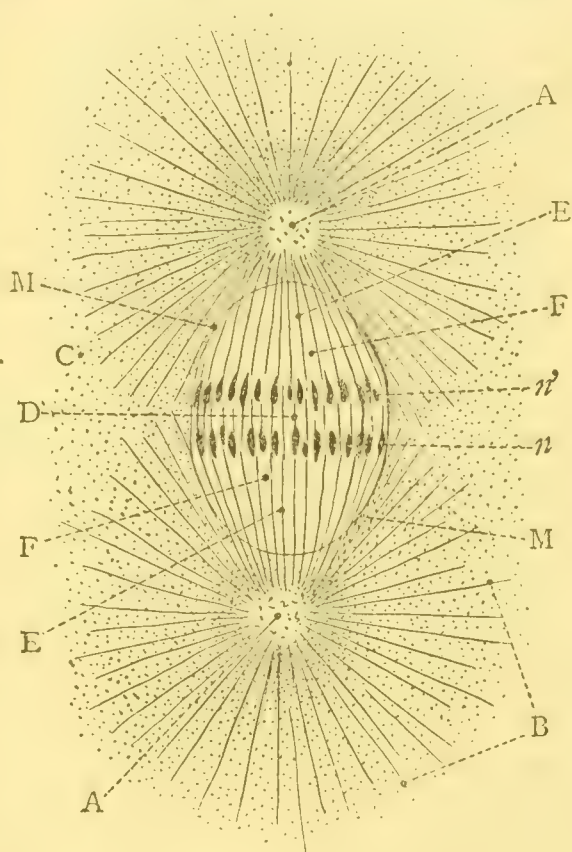


Figure V. — Loligo.

identical by the means of observation at our disposal. Thus, instead of inferring function from structure, we infer structure from function, and conclude that wherever we detect a difference in function the protoplasmic structure must be different also. When, therefore, we speak of the analysis of nuclear substances we do not speak from actual knowledge of the substances thus analyzed, but from purely scientific reasoning.

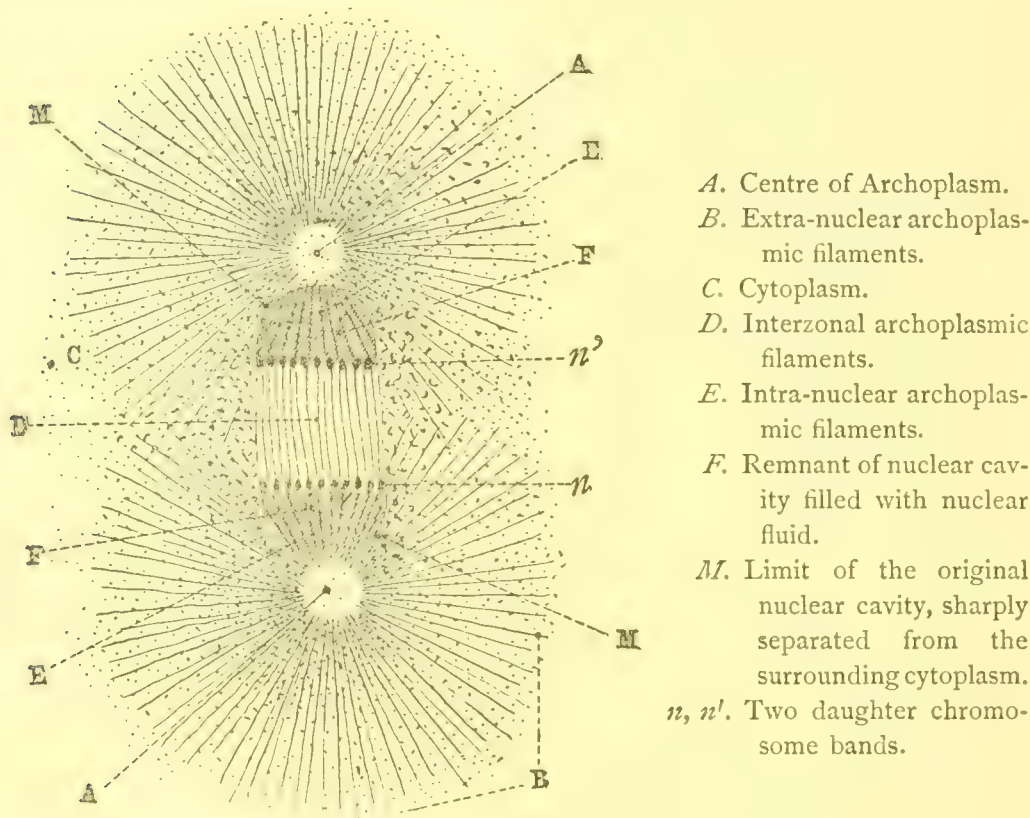


Figure VI. — Asterias.

It is probable that during cleavage, the original nuclear substance may undergo a series of molecular changes, and split up into a number of protoplasmic substances, each of a different molecular structure, and that as a final result of this chain of metamorphoses different kinds of tissue-cells come into existence.¹ In short, different morphological stages of the developing ovum may be considered as so many different molecular conditions of the protoplasm. And perhaps the molecular constitution of a dividing ovum in its earlier stage may differ more from that of the later larval stage, than two organisms

¹ See in this connection, Roux: *Bedeutung der Kerntheilungsfiguren*. 1883.

belonging to different species would differ from each other in their adult condition. Professor Weismann's phrase — "ontogenetic stages of idioplasm" — aptly expresses our meaning on this subject. For the metamorphoses of structures and of embryonic tissues must of necessity correspond to the change in the constituent protoplasm. Without change in the nuclear substance, development is impossible; the egg must remain an egg forever.

If all the determining elements of future tissues are contained in the nucleus of the ovum, and if cleavage is the process by which these elements are analyzed into more tangible tissues, the question naturally arises as to the method of analysis employed in such a process.

Such a *method* we find in *Caryokinesis*.

I will, therefore, describe the process which may be termed the mechanics of nuclear division, as based on my observations on Cephalopods and Echinoderms.¹

It is now agreed by many foremost investigators of the subject that the essential feature of caryokinesis lies in the division of the chromatic substance of the nucleus among the daughter cells, and that the complicated system of spindle rays is the mechanism to effect such a division. The development of a spindle clearly shows this, and the following is an attempt towards a further confirmation of the current view on the subject, as held especially by E. van Beneden and T. Boveri. In one important respect my view is just opposite to that of these authors, but this difference lies more in the interpretation of phenomena than in the facts themselves.

First of all, I will endeavor to describe the anatomy of a well-developed caryokinetic figure in the Cephalopod egg, upon which my observations have been chiefly carried on. The question of nomenclature presents some difficulty. I will use here a set of terms of a simple descriptive character, descriptive of function, of origin, or of topographic relationship of different parts.

The accompanying illustration, Fig. V, shows a caryokinetic

¹ The substance of this portion has been given in my two previous notes on the subject: *Karyokinesis and the Cleavage of the Ovum*, Johns Hopkins Univ. Circulars, April, 1890; *On Caryokinesis*, Biological Lectures delivered at the Marine Biological Station, Wood's Holl, 1891, pp. 168-187.

figure in the blastoderm of the squid. Fig. VI shows the same in a more advanced condition, as seen in the developing ovum of a starfish (*Asterias vulgaris*) common at Wood's Holl. The latter was brought out by the application of the method recommended by Boveri in his recent paper.¹ The figure shows a striking resemblance to those given by Agassiz and Whitman² in their recent work on the development of the osseous fishes.

The figure consists of two essential anatomical features, (1) the central, elliptical body, and (2) the two star-like, radiating structures. The former corresponds to the outline of the original nucleus, as will be shown later, and the latter constitute the *asters* of Fol, *sphères attractives* of van Beneden, or, to use a more recent nomenclature, the *archoplasmic spheres* of Boveri. The central area of archoplasm (*A*) is situated in the substance of the *cytoplasm* (*C*). From the granular archoplasmic substance as a centre, there radiate out in all directions a large number of fibre-like rays, the *archoplasmic filaments* (*B*) and (*E*). A portion of these ray-fibres penetrate into the elliptical part of the figure, and constitute the *intra-nuclear archoplasmic filaments* (*E*); while those lying outside of the elliptical body are the *extra-nuclear archoplasmic filaments* (*B*).

The elliptical portion of the figure consists of three parts, two terminal and one intermediate. The terminal portion, which presents different optical properties from the intermediate part, consists of a hemispherical mass of a slightly stainable, semi-liquid substance, which I believe to be the nuclear sap of the original nucleus. Into this part the archoplasmic rays extend, as has already been mentioned. The two terminal masses of stainable substance are separated from the intermediate non-stainable bundle of filaments by parallel chromatic "plates" (*n'*), (*n*), — the *chromosomes* (Waldeyer) of the original nucleus. The non-stainable intermediate filaments above referred to are the *interzonal archoplasmic filaments* (*D*), — "interzonal filaments" of Mark, "filaments réunissants" of van Beneden, "gubernaculum" of Maupas, "Verbindungschlauch" of Strasburger, "connective filaments," "Verbindungsfäden," etc., of authors.

One plate of chromosomes goes to one daughter nucleus, and

¹ *Zellen Studien*, III, pp. 6, 7, 1890.

² Agassiz and Whitman: *Memoirs of the Museum of Comparative Zoölogy at Harvard College*, Vol. XIV, No. 1, 1889.

the other to another. The cytoplasm accumulates around each, and there follows a separation into two cells, each with its distinct nucleus.

If one examine a nucleus at a tolerably early stage of caryokinesis, one will see a phenomenon such as is shown in Fig. VII. The nucleus with a network of chromosomes is intercepted between two archoplasmic spheres. More than this, however. That portion of the archoplasmic rays which falls on the surface of the nucleus presses that part inward and so flattens that side of the nucleus. This polar flattening of the nucleus goes on until the nucleus presents the appearance shown in Fig. VIII.

Space only forbids the illustration of the further changes, but it may be easily imagined that when this flattening of the nucleus is continued, the whole solid contents of the nucleus are reduced to a single flat sheet, as it were, as shown in Fig. IX, forming the equatorial chromatic "plate." The spindle, then, as its history clearly indicates, consists of two cones with their bases turned toward each other, and with their apices in the archoplasmic centres, as was first pointed out by van Beneden.

This stage of caryokinesis with its single chromatic "plate" leads to another with two daughter "plates," — a phase which has been called by Flemming, metakinesis.

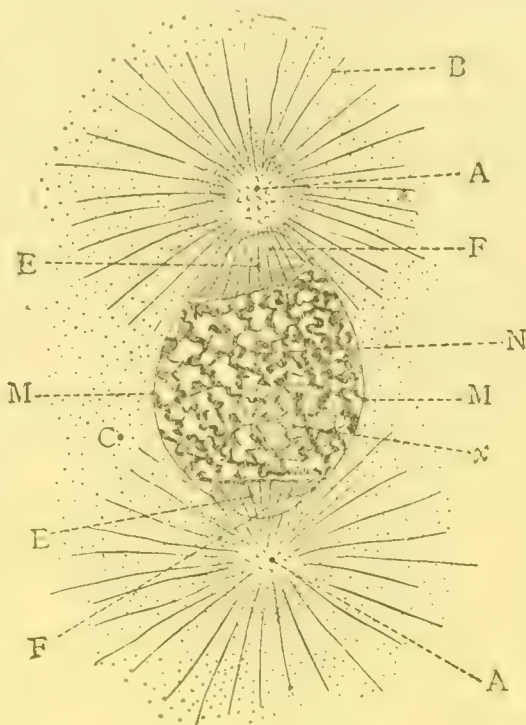


Figure VII. — *Loligo*.

N — Nucleus, α , nucleolus (?).

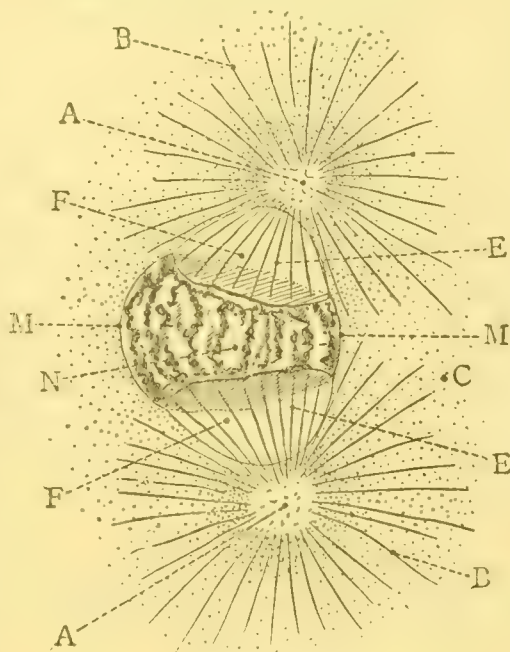


Figure VIII.

The question naturally arises, How is this separation of a single "plate" into two "plates" effected? With the separation of the two daughter "plates" of chromosomes, there comes into existence a series of parallel interzonal filaments which lie between the two separating "plates." The separation of the daughter "plates" of chromosomes, and the formation of the interzonal filaments, are so intimately connected with one another that we naturally look for a causal connection which

underlies the parallel series of phenomena. Any theory which explains the one must also explain the other.

Before I venture a suggestion in regard to this important point, it will be important to find out whether the phenomenon of polar flattening of the nucleus, such as I have described in the previous pages, is one of normal character or not. That the said phenomenon is of a normal character is shown by its constant occurrence in the blastoderm of the squid, where all the differ-

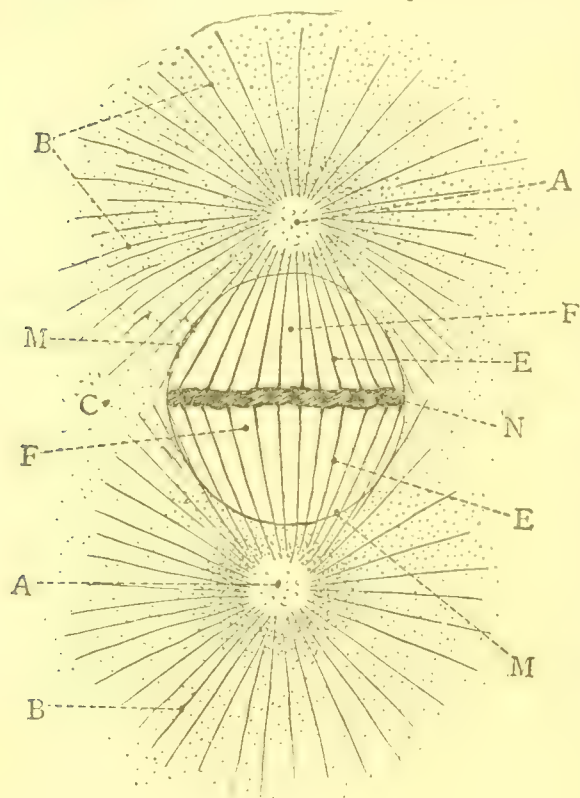


Figure IX.

ent stages of caryokinetic division can be observed without any difficulty. My recent studies on the eggs of a starfish (*Asterias vulgaris*), with this particular point in mind, convinced me that there, as in the squid, the polar invasion of the nucleus by the archoplasmic filaments is the matter of normal occurrence. This phase, however, is extremely transient, and quickly passes into the equatorial plate stage.

In connection with the caryokinetic phenomena in *Pagurus striatus*, Carnoy¹ observed the formation of the equatorial chromatic plate by the "gradual retraction of the chromatic loops

¹ Carnoy: *Cytodiérèse chez les Arthropodes*, Pl. VII, Fig. 44, a, b, c, d, f, p. 316.

towards the equator" of the nucleus. In the first stage, the nucleus retains the elliptical outline, with a complete nuclear membrane, and two archoplasmic asters at the two antipodal ends of the nucleus. In the second stage, the retraction of the nuclear filaments towards the equator has commenced, and with it, the rudiment of the archoplasmic spindle appears inside of the nuclear membrane, presenting very much the same appearance as in Fig. VII, p. 267, previously described. This retraction towards the equator goes still further in the third stage, and with it the shape of the spindle becomes more perfect. In the fourth stage, the nuclear filaments are reduced into a plate-like structure, and the original cavity of the nucleus, still sharply bounded by the nuclear membrane, contains the complete spindle; and in the succeeding figure (*f*) the metakinesis in its advanced stage is represented. The resemblance of this series of figures to the series I have already given is perfect.

These observations, according to Carnoy, show two important facts:—

(1) The persistence of the nuclear membrane through the stages of caryokinesis.

(2) The developmental history of the spindle. After all this, Carnoy,¹ however, concludes, "Ils prouvent d'abord que le fuseau peut dériver exclusivement du noyau."

It appears to me to say that the spindle is formed inside of the nuclear membrane is one thing, but it is quite another to infer from it that the spindle is derived from the nucleus. Why may it not be formed by the invasion of the archoplasmic filaments from the outside of the nucleus, through the porous nuclear membrane, as was pointed out by Strasburger and Guignard?

I am strongly inclined to believe that the interesting series of stages in the formation of the spindle and of the chromatic equatorial "plate," given by Carnoy in the place above referred to, belongs to the same order of facts presented in the previous pages in connection with the origin of the spindle in the Cephalopod blastoderm. May not the beautiful series of figures given by Rabl² and Schewiakoff,³ showing the gradual formation of

¹ *loc. cit.* p. 317.

² Rabl: *Ueber Zelltheilung*. Morph. Jahrbuch., Bd. X.

³ Schewiakoff: *Ueber die Karyokinetische Kerntheilung der Euglypha alveolata*. *Ibid.*, Bd. XIII.

the spindle with the corresponding retraction of the chromatic filaments toward the equator of the nucleus, show the same thing, and that the spindle material does not originate inside of the nucleus, properly speaking, but comes from the outside?

The most convincing facts in regard to the truth of the polar invasion of the archoplasmic filaments, in the nucleus with the firm nuclear membrane around it, are afforded by Bütschli's¹ observation in the living egg of *Branchipus*, by Mark² in *Limax*, by Platner³ in *Aulostoma*, and by Garnault⁴ in *Helix*. Further, I believe that the facts given by these naturalists have the same significance in the course of caryokinetic phenomena, which I have ascribed to them in the preceding pages, in the case of Cephalopods.

To van Beneden's works references have already been made in the preceding pages. The following is the summary of his more recent results in his studies on the mechanics of caryokinesis in *Ascaris megalocephala*, which he carried out in conjunction with A. Neyt:—⁵

“Dans notre opinion,” van Beneden and Neyt observe, “tous les mouvements internes qui accompagnent la division cellulaire ont leur cause immédiate dans la contractilité des fibrilles du protoplasme cellulaire [archoplasmic fibrils] et dans leur arrangement en une sorte de système musculaire radiaire, [archoplasmic sphere] composé de groupes antagonistes; le corpuscule central joue dans le système le rôle d'un organe d'insertion. Des divers organes de la cellule c'est lui qui se divise en premier lieu, et son dédoublement amène le groupement des éléments contractils de la cellule en deux systèmes ayant chacun leur centre. La présence de ces deux systèmes entraîne la division cellulaire et détermine activement le cheminement des étoiles chromatiques secondaires dans des directions opposées. Une partie importante des phénomènes qui constituent la cinèse a donc sa cause efficiente, *non dans le noyau, mais dans le corps protoplasmique de la cellule.*

¹ O. Bütschli: *Studien über die ersten Entwicklungsvorgänge der Eizelle, die Zelltheilung und die Conjugation der Infusorien.* 1875. Taf. XIII, Fig. 14.

² Mark: *Maturation, Fertilization, Segmentation of Limax*

³ Platner: *Arch. f. Mik. Anat.*, Bd. 33.

⁴ Garnault: *Bull. Scientifique de la France et de la Belgique*, Tome XXII.

⁵ E. van Beneden and A. Neyt: *Nouvelle recherches*, etc., p. 280. (*Italics are my own.*)

“D’où vient l’impulsion qui détermine le dédoublement des corpuscules centraux, la formation des cordons pelotonnés et la division longitudinale des anses? Réside-t-elle dans le noyau, ou dans le corps cellulaire? Aucune donnée positive ne permet de résoudre cette question. Nous n’avons réussi à établir que deux choses: *c’est l’existence dans la cellule d’un appareil ou d’un mécanisme qui préside à la division cellulaire, comme notre système musculaire à la locomotion, et le dédoublement de ce mécanisme préalablement à la division nucléaire.*”

To this must be added his account of the origin of interzonal fibrils. As van Beneden¹ observes, with the division of the equatorial chromosomes and their respective migrations towards opposite poles, there comes into existence, intercepted by two daughter “plates,” a lamina, which he calls the “*lame intermédiaire*,” and the substance which composes it, the “*substance intermédiaire*” (p. 543).

This substance is described as being “relativement sombre et chromophile, mais se colorant de moins en moins au fur et à mesure que l’écartement [of daughter chromatic stars] progresse” (p. 556).

As the separation of the chromatic loops progresses, one can positively ascertain the existence of achromatic fibrils in this intermediary substance. To these achromatic fibrils van Beneden gave the name of the “*filaments réunissants*,” to distinguish them from the achromatic spindle fibrils directly arising from the *sphères attractives* (pp. 557, 558).

As to the origin of the entire achromatic spindle, van Beneden considers it as highly probable that it is derived from two sources, a part from the *sphères attractives* (cytoplasmic in their origin), and a part from the achromatic part of the nucleus.

The achromatic constituent of the nucleus contributes to the formation of the *substance intermédiaire* with its fibrils, while the ends of the spindle are derived from a portion of the attractive spheres. The gradual lengthening of the achromatic fibrils in the interzonal space is due to the antagonistic pulling forces emanating from the attractive spheres which act through the contractile fibrils whose distal ends are attached to separating

¹ E. van Beneden: *Recherches sur la maturation de l’œuf et la fécondation*. Archives des Biologie, Tome IV, 1883.

daughter stars. For the graphic illustrations of this view, the reader is referred to the diagrams constructed by Boveri.¹

Boveri's² view on the origin of the interzonal filaments in *Ascaris* is a remarkable one. He agrees with van Beneden in regarding the interzonal fibrils as essentially different from the archoplasmic spindle fibres. In fact, Boveri does not regard the interzonal filaments as filaments at all, but simply as the optical expression of longitudinal folds of the "*lamelle intermédiaire*" of van Beneden, brought about by the contraction of the archoplasmic fibrils, whose distal extremities are fastened to the chromosomes of the nucleus, and the consequent stretching of the intermediary lamina in the longitudinal direction of the spindle. The folds thus produced run parallel with the longitudinal axis of the caryokinetic figure, and give rise to the filamentous appearance. Boveri admits that the interzonal filaments in the caryokinetic figures in other cells, demand a different explanation from that given above.

It would be entirely out of place here to enter into the examination of the details of the phenomena so admirably brought out by Boveri. But before leaving Boveri I would like to call attention to his Fig. 41,³ Taf. XX, in the above-mentioned memoir, in which a group of archoplasmic threads from the upper centre fasten themselves to two chromatic loops in common with somewhat longer archoplasmic threads arising from the opposite centre.

The figure suggests the idea that the upper bundle of archoplasmic threads are yielding to the greater pushing force of the lower. If the archoplasmic fibrils are contractile in movement during the stages of metakinesis, and are pulling the chromosomes towards their own archoplasmic centres, how does it happen that this bundle shows such a peculiar curvature, its convex surface being turned away from the centre which is supposed to be the centre of attraction?

Another point I would like to observe in connection with the theory of van Beneden and Boveri on the function of the archo-

¹ Boveri: *Zellen-Studien*, II. Jenaische Zeitschrift, Bd. XXII, Taf. XXI, Figs. 64 a, 64 b. See, also, Geddes and Thompson: *The Evolution of Sex*, p. 222. London, 1889.

² Boveri: *loc. cit.*

³ Reproduced in Geddes and Thompson: *The Evolution of Sex*, p. 146, Fig. 3.

plasmic fibrils, and that is the duplex activity of the archoplasmic threads at two different periods of caryokinesis. In the first half of the process, the fibrils are distensible, stretching away from the archoplasmic centres, and in the latter half, the activity becomes just reversed and then become contractile, travelling towards the respective centres from which they arise.

Strasburger has always held that the archoplasmic spindle threads have a cytoplasmic origin, the threads penetrating the nucleus from two poles, through the porous nuclear membrane. In his more recent studies on *Spirogyra*,¹ he conclusively supports his former conclusion.

As to the origin and significance of the interzonal fibrils, Strasburger says:—²

“Augenscheinlich sammelt sich zwischen den beiden Kernplattenhälften ein osmotisch wirksamer Stoff an, der Zellsaft aus der Umgebung an sich zieht und der die angrenzende Plasmahülle nach aussen treibt. Es lässt sich annehmen, das dieser Stoff zuvor zwischen den Spindelfasern angesammelt war; ja ich möchte noch weiter gehen und die Vermuthung aussprechen, das derselbe dem ursprünglichen Kernsaft entstammt.” Throughout the process of nuclear division the nucleus is enclosed by a cytoplasmic mantle. As the two daughter nuclei separate wider and wider, the equatorial achromatic space lying between them assumes the swollen barrel shape, which is enveloped by an extremely thin cytoplasmic bag. This interzonal structure is designated as the *Verbindungsschlauch*, in which the equatorial suspensory threads appear prominent as pronounced ribs. This “*Verbindungsschlauch*” constantly increases in length and breadth by osmotic absorption of fluid through its walls. Strasburger offers the following suggestion in regard to the significance of this structure: “Es unterliegt kaum einem Zweifel, das wir in dem Verbindungsschlauche eine Einrichtung vor uns haben, die mit Zuhülfenahme osmotischer Druckkräfte bei *Spirogyra* dazu dient, die Zellkerne auseinander zu treiben, sie in bestimmter gegenseitiger Lage zu erhalten, endlich den Ausschluss der gesammten Theilungsfigur an die vordringende Scheidewande herzustellen” (pp. 21, 22).

Strasburger, as has been seen, regards the interzonal filaments

¹ *Ueber Kern- und Zelltheilung in Pflanzenreiche*, etc. 1888.

² *loc. cit.* p. 17.

as first arising from the achromatic portion of the original nucleus, to which, by osmotic action, is added the substance from the surrounding cytoplasm.

While Strasburger regards the "Verbindungsschlauch" as a mechanism for separating the two daughter chromatic "plates" apart, he also ascribes to the latter a certain automatic capacity for movement. The chromatic threads may probably use the spindle filaments as supports in the course of their migration. Strasburger also supposes that the poles of the spindle exert a certain influence upon the behavior of the chromatic threads. "In welcher Art man sich die Wirkung der Pole denken mag, kommt dabei zunächst nicht in Betracht, es wäre ja möglich, dass es sich dabei um einen chemischen Reiz handelt, ähnlich demjenigen, der die Plasmodien, Bakterien oder Oscillaria-Fäden veranlasst, bestimmte Bewegungsrichtungen einzuschlagen" (p. 153).

Strasburger then recognizes four possible factors which determine the movement of the chromatic threads: (1) Spindle fibres, cytoplasmic in origin, penetrating the nucleus, through the nuclear membrane; (2) the "Verbindungsschlauch," partly nuclear, partly cytoplasmic in origin, driving the two daughter chromatic plates wider apart towards the poles of the spindle; (3) the chromatic granules forming the nuclear threads having an automatic power of movement, and travelling themselves towards the poles, using the achromatic spindle fibres as supports; and (4) the poles may exert a certain influence upon the migrating chromatic threads, whose nature is difficult to imagine, but may possibly be chemical in its nature.

There are a few more theories recently published, attempting to explain the phenomena of caryokinesis on a mechanical ground. The main object of the present paper not being to discuss caryokinesis, I cannot enter into a fuller examination of different views on the subject at present.

To turn again to the description of the caryokinetic process in Cephalopod. As to the filamentous nature of the interzonal substance, there can be no question, as several observers have abundantly shown. My own studies on Cephalopods and Echinoderms have convinced me of the truth of this conclusion. Further, no optical difference could be observed between the archoplasmic fibrils at the poles of the spindle and the filamen-

tous bodies in the intermediate zone, which fact has already been pointed out by several investigators.

Observing, then, (1) that the interzonal portion of the caryokinetic figure consists of the bundle of filamentous substance, (2) that this filamentous substance is essentially the same as the archoplasmic filaments of the spindle, (3) that the length of these filaments is exactly the same as the space between the parallel bands of chromosomes in all stages, (4) that the archoplasmic filaments have been growing in length from the poles toward the equator of the nucleus, and (5) that the interzonal filaments came into existence exactly at the moment when the single equatorial "plate" was dividing into two parallel daughter "plates," the following view becomes probable, viz. that after the archoplasmic filaments from the two centres have reduced the chromatic contents of the nucleus into a flat "plate" by gradual lengthening, they continue to grow in the same manner, and push through between each other, just as two brushes would do if their ends were pushed together. Their free ends dovetail with each other. The distal extremity of each archoplasmic filament being fastened to the chromosome, the latter will be carried by the former at its tip, and will be pushed forward as long as the filament continues to grow. Two opposing systems of the archoplasmic filaments behaving in a similar way, and lengthening in a contrary direction, would reduce the spherical nucleus first to a biconcave disc, then to a flat "plate," and finally, into two parallel "plates," each "plate" travelling in an opposite direction. The interzonal filaments then, according to this view, are the actual continuations of the archoplasmic filaments; but, instead of consisting of a single system, as at either end of the spindle, they are composed of two systems, each dovetailing with the other, and growing in contrary directions. *Interzonal filaments* are, therefore, the prolongations of the *intranuclear filaments*. I am further inclined to believe that a certain optical peculiarity of the interzonal region, as, for instance, its aversion to take stains, is due to the existence in it of a proportionally large number of non-stainable archoplasmic filaments.

Having briefly sketched the general outline of the process by which the characteristic shape of a caryokinetic figure originates, it would be appropriate to devote a few words to the obscure

point as to the origin and movement of the archoplasm itself. But as a matter of fact, we know as yet very little in regard to the origin of the archoplasm, which has sometimes a definite body in its centre—the *centrosome*. A great authority like van Beneden looks upon it as a permanent organ of the cell, equal in value to the nucleus itself; but the whole question of its origin and its apparent homologues, which pass by different names in different cells, is too complicated and obscure to be discussed in this place.

The later history of the archoplasm is, however, better known. When we examine a cell at the close of caryokinetic division, we see a small nucleus with the archoplasmic sphere at one side of it, appearing somewhat like a satellite of a planet. This small nucleus is one of the daughter nuclei of the previous generation, and is destined to become the mother nucleus of the next. Just as new nuclei arise by the division of the old one, so the new archoplasmic spheres also arise by the division of the previous one. In the Cephalopod blastoderm the division of the mother archoplasmic sphere into two daughter spheres could be observed with sufficient clearness. In *Ascaris*, its division has been most carefully studied by several investigators. At first the two daughter spheres lie in close opposition; later, they separate more and more widely. As each sphere has a system of radiating filaments, there is formed a little spindle where they come into contact. This spindle lies outside of the nucleus, and has nothing to do with the larger one which has been described already. The daughter archoplasmic spheres migrate further apart, and finally settle themselves on the opposite sides of the nucleus. Their effect on the latter is soon seen. That surface of the nucleus upon which the archoplasm rests soon shows signs of flattening, as was shown in Fig. VII. This polar flattening of the nucleus has been interpreted as due to the pressure exerted by the growing archoplasmic filaments. The growth of the filaments continues, and the effects it produces upon the nucleus, in the arrangement and distribution of the chromosomes, have already been described. Compare in this connection the series of stages shown in Figs. VII, VIII, IX, and V.

The above is a brief sketch of the mechanics of nuclear division, as I interpret them from the study of Cephalopod

blastoderm. The descriptions of the different stages, which are but summarily given in connection with the few diagrams, will be supplemented in a future paper on the caryokinesis in Cephalopods and Echinoderms.

The preceding descriptions of the phenomena of caryokinesis in Cephalopods refer to four important topics : (1) the origin of the spindle ; (2) its behavior towards the nucleus ; (3) the formation of the equatorial chromatic "plate" ; and (4) the separation of the daughter plates and the formation of the interzonal filaments.

I have attempted to show that these series of phenomena are the continuation of one and the same process, with no reversal of activity in the middle, nor with the introduction of several hypothetical factors.

The spindle, however, plays only a part in the production of the caryokinetic phenomena. The whole behavior of the chromosomes preparatory to division, such as the transformation from a resting condition to a coil stage, followed by the longitudinal splitting of each filament, — phenomena which take place independently of the influence of the spindle, — has received no consideration, and, so far as I can see, has no causal connection with the behavior of the archoplasm, although both tend to accomplish the same end, viz. the formation of two nuclei out of one. It is conceivable that one mother coil may sometimes split into two different kinds of substances, and the archoplasmic filaments play simply the part of a distributing agent in carrying these into opposite halves of the dividing cell. In view of the general theoretical conclusion regarding the intimate correlation between form and matter, and mechanism and function, such a view does not appear improbable ; for, as has already been stated, the differences of two cells lie in their structure, and the structure being the expression of the chemical substance of the protoplasm which composes them, wherever we find the difference of structure we find difference of substance or substances, and wherever we find difference in the substance we find difference in property or function. It is probable, as has been mentioned already, that the nuclear substance, by its constant metamorphoses, gives rise to a series of substances, to isolate and distribute which is the function of the spindle, a number of differently constituted cells being thus produced.

If, in conclusion, I recapitulate what has been said in a few words, the cleavage of the ovum may be characterized as *Analysis* of different protoplasmic substances which form the bases of different tissues, caryokinesis as the *Method*, and the archoplasmic spindle as the *Instrument*.

IV.

Since the work of Kölliker was published in 1844, there have appeared a number of valuable observations on the development of Cephalopods, touching more or less on the cleavage of the ovum, such as those of Bobretzky, Lankester, Ussow, Brooks, and Bruce, and quite recently an important contribution on the cleavage in *Sepia* by Vialleton.

So far as the observation of facts are concerned, there is very little chance for error in forms like the Cephalopods. Differences of methods, so far as they come within the limits of approved histological practice, can make but a little difference, particularly in the study of such a subject as this, where everything comes out with a diagrammatic clearness. In regard to the illustrations of the different stages in *Loligo Pealei* which accompany this paper, I feel confident of their general correctness, as far as they go. Most of the preparations from which the illustrations on my plates were drawn are still in existence, and can be verified at any time. I will not, therefore, enter into a critical comparison of the facts presented by different authors. In regard to the interpretation of the phenomena, differences are naturally expected. Whatever may be the value of the preceding remarks concerning the cleavage of the ovum in general, I will attempt to treat the cleavage phenomena in *Loligo* from that standpoint. In this point my paper differs from that of any previous worker on the Cephalopod embryology.

Before we enter into the descriptive details of the present subject, a few points of a more general nature occur to me, which may be introduced here.

The idea of ascribing a comparatively high, complex structure to the ovum, such as the differentiation of the axes, etc., in the unsegmented egg is attended with scepticism, or accepted with a certain amount of reserve by more cautious embryologists. It appears to me, this hesitation arises chiefly from the prevalent

idea that the ovum of a metazoan represents phylogenetically a protozoan which is supposed to have possessed a radial symmetry. We must, however, bear in mind that the ovum at the moment of its first separation as a single cell in the then embryonic body of the parent organism, is different from the ovum at the stage ready for cleavage. If by the growth of an organism is meant an increase in the volume of the original protoplasm and the development of the external configuration, and the cleavage of its material is regarded as a secondary feature which may be dispensed with in the strict definition of the term as applied in the organic kingdom, then the history of the ovarian ovum is just as much a growth in all of its essential features as the conversion of the segmented ovum into an embryo, and the embryo into an adult organism.

The ovarian ovum increases in size; it acquires protective apparatus around it; it develops a particular external configuration; and, above all, it accumulates a greater or less amount of yolk-substance for future consumption. The ovum at the time it is ready for cleavage is not the same organism as when it was first formed inside of the maternal tissues, any more than the two segment stage of the same ovum is the same organism as it is at an advanced stage of its ontogeny. There is a growth and development in the unicellular phases of the one, as there are growth and development in the polycellular phases of the other. When, therefore, one says that the ovum of a metazoan is the phylogenetic representation of its protozoan ancestry, we may ask whether one refers to the primitive ovum first found in the embryo, or to one advanced to the stage ready to divide, between which, we must remember, there exist various stages of growth and development.

From the developmental standpoint the origin of the ovum is just as old as many of the functional tissues of the parent organism. It is true that the growth of the ovarian ovum is an extremely slow process compared with other tissue-cells, but, nevertheless, it is a growth, and a continuous one, and directly depends on the nourishment and protection afforded by the tissues of the parent organism. Even if we admit that the unicellular ovum, irrespective of its stages of growth, represents actually the condition of the ancestral protozoan, a highly differentiated axial symmetry of a certain metazoan ovum cannot

be said to be an aberrant feature unrepresented in the ancestral protozoa, so long as the existing forms of the protozoa often show such a high degree of differentiation in that particular respect.

It appears to me admissible to say at present that the ovum, which may start out without any definite axis at first, may acquire it later, and at the moment ready for its cleavage the distribution of its protoplasmic substances may be such as to exhibit a perfect symmetry, and the furrows of cleavage may have a certain definite relation to the inherent arrangement of the protoplasmic substances which constitute the ovum. Hence, in a certain case, the plane of the first cleavage furrow may coincide with the plane of the median axis of the embryo, and the sundering of the protoplasmic material may take place into right and left, according to the pre-existing organization of the egg at the time of cleavage; and in another case the first cleavage may roughly correspond to the differentiation of the ectoderm and the endoderm, also according to the pre-organized constitution of the protoplasmic materials of the ovum.

It does not appear strange, therefore, that we may detect a certain structural differentiation in the unsegmented ovum, with all the axes of the future organism already foreshadowed in it, and the axial symmetry of the embryonic organism identical with that of the adult.

The general shape of the ovum of the squid at the time it is ready for cleavage is oblong, having the shape of a hen's egg, with one end more pointed than the other (Fig. I, Pl. IX). On the pointed end is situated the germ-protoplasm, spreading its thin pellicle over the huge mass of the food-yolk and completely enveloping it. The germ-protoplasm at the pointed pole shows such a thickening, that, when viewed from the side, it presents a lenticular outline, sharply distinguished from the underlying mass of food-yolk (Fig. X). The polar globules, three in number, one a little larger than the rest, are seen floating in the perivitelline fluid inside the chorion.

By keeping the exact position of the segmentation nucleus and the outline of the whole germ-disc as shown by the optical section in view, and rolling the egg in its longitudinal axis, we observe two important facts. At one time we observe a slight inequality in the amount of cytoplasm on both sides of the

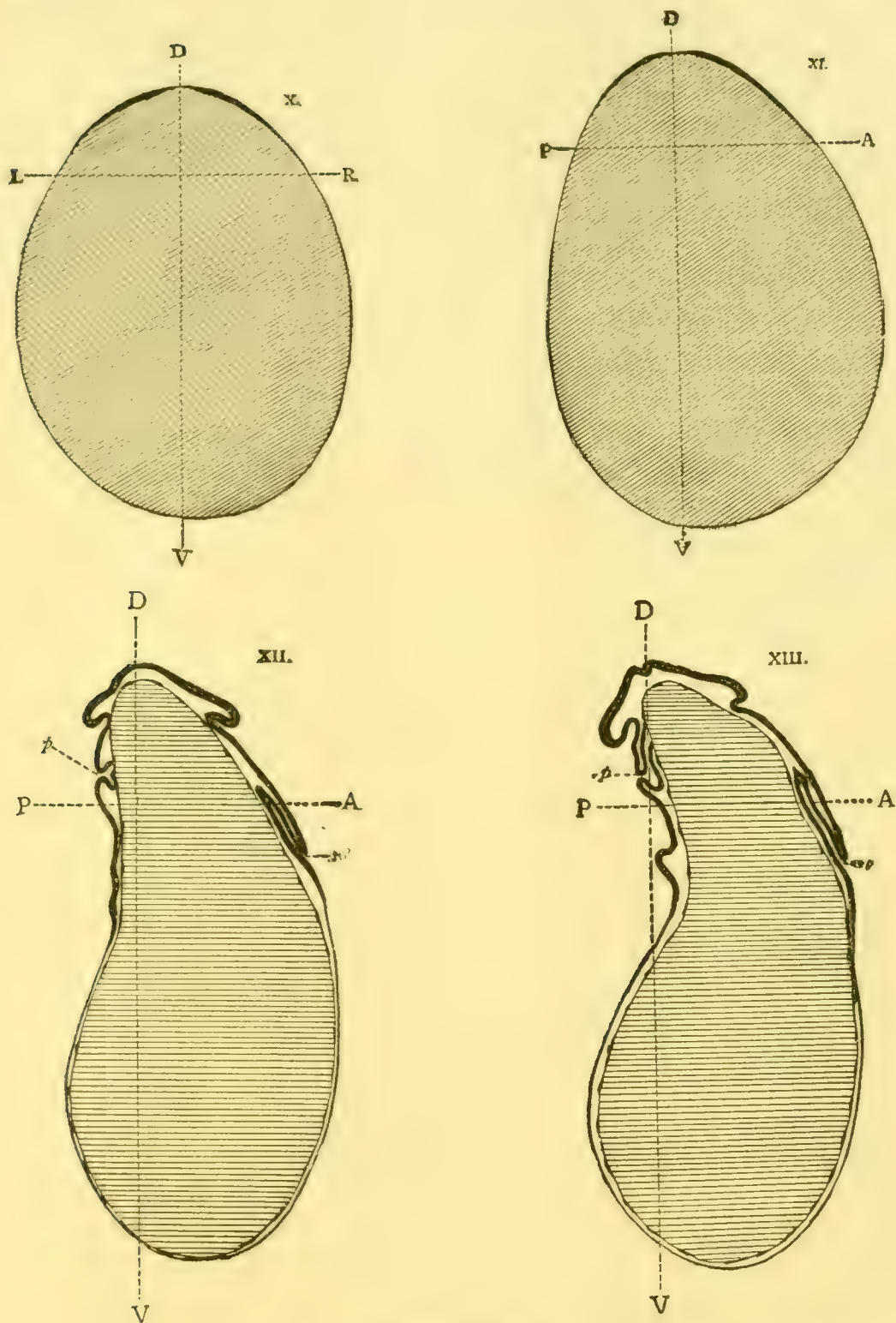
*Loligo Pealei.*

Fig. X. — An optical section of an unsegmented ovum. The optical axis coincides with the antero-posterior axis of the ovum.

Fig. XI. — A profile view of an unsegmented ovum.

Figs. XII and XIII. — Side views of squid embryos at two stages of development. The flexure of the body corresponds, in the main, with the outline of the unsegmented ovum (Fig. XI), A, anterior; D, dorsal; P, posterior; *p*, proctodæum; *s*, stomodæum; V, ventral.

polar extremity (Fig. XI), and at the next moment the wings, as it were, of the cytoplasm on both sides of the segmentation nucleus become perfectly identical in outline (Fig. X). By rolling the egg, we are constantly brought to view the same alternation of facts with perfect regularity.

Coupled with these phenomena is another presented by the general outline of the ovum. When the wings of the cytoplasm on both sides of the segmentation nucleus appear unequal (Fig. XI), then the whole outline of the ovum corresponding to the above is also unequal, the longer arm of the germ-wing being accompanied by the broader, convex outline (A), while the side corresponding to the shorter wing of the cytoplasm descends rather abruptly (P). By a further study of the phenomena through successive stages of development, it can be demonstrated that this more convex border corresponds to the anterior, and the less convex border corresponds to the posterior, side of the egg (Figs. XII, XIII). The view (Fig. X) in which the wings of the cytoplasm are equal is the one in which the optical axis of observation coincides with the antero-posterior axis of the ovum; hence the two wings correspond to the right and the left sides of the organism (R, L, Fig. X). The view in which the distribution of the cytoplasm around the nucleus is unequal is the one in which the optical axis of observation coincides with the transverse axis of the ovum (Fig. XI). The longer wing of the cytoplasm corresponds to the anterior border of the ovum (A), and the shorter wing corresponds to the posterior border of the same (P). Thus in the unsegmented ovum of the *Loligo*, the surface study alone indicates that there is an *anterior border* (A), different from the *posterior* (P), and with it there is a *right* (R) and *left side* (L) of the organism. As the later embryological studies abundantly show, the end where the germ-protoplasm is situated corresponds to the *dorsal side* (D), and the opposite pole, where the food-yolk predominates, is the *ventral side* (V) of the organism.

This surface observation, when combined with the study of the blastoderm completely separated from the bulk of food-yolk, makes the point still more clear. Such a specimen is shown in Fig. 16, Pl. X. The germ shows four well-marked concentric zones. The segmentation nucleus occupies the innermost circle. Next around it comes a zone of clear substance in

which the achromatic radiating fibrils abound. Next to this is the zone of granular cytoplasm which passes rather suddenly into the zone of the thinner protoplasmic pellicle, a portion of which is shown in the figure. The whole mass of the yolk-substance is enveloped by the extension of this same pellicle.

A further study of the same blastoderm will show that the nucleus is not situated exactly in the centre of the germ-disc, but it is slightly eccentric. Taking the outermost limit of the granular zone as the contour of the essential part of the developing egg, the cleavage nucleus is found situated nearer to one side than to the other. I have made a series of careful measurements in regard to this point, and found in a large majority of cases, this eccentric position of the nucleus in the blastodisc is well pronounced, and even discernible without any aid of a micrometer. This fact was already noticed by Vialleton in *Sepia*. Comparing this observation on the isolated blastoderm with the surface study, we notice an interesting coincidence of the two. For if we draw a straight line through the cleavage nucleus, transverse to the germ-disc, as it is represented in the plate, it will divide the latter into two unequal halves, the one lying in front of the line being larger than the one lying behind it, while if we draw a straight line at right angles to the first and through the same nucleus, it will divide the disc into two identical bilateral halves. The larger segment lying in front of the transverse line corresponds to the anterior half, and the smaller segment behind the line corresponds to the posterior half of the ovum. The two bilateral identical segments falling on both sides of our second imaginary line correspond to the right and the left half of the ovum.

Bearing these points in mind, we will proceed to the examination of the different stages of cleavage, with special reference to the development of symmetry as manifested by the cleavage furrows.

Fig. 17 shows a stage in which the caryokinetic figure of the cleavage nucleus has just reached a stage where the chromatic contents of the nucleus have been reduced into a "plate," and the general outline of the spindle has become completed. The spindle is formed entirely within the original nuclear cavity, which is sharply separated from the cytoplasmic surrounding. The archoplasmic rays of the aster run in all directions, and

some of them meet with the rays of the opposite side in the plane of the equatorial chromatin "plate." This plane corresponds to the median axis of the ovum.

Fig. 18 represents the blastoderm with the first furrow of cleavage completed. The furrow is deepest in the area where the layer of cytoplasm is thickest, becoming gradually shallower and shallower until it finally disappears in the peripheral zone of the blastoderm, or in the zone of the protoplasmic pellicle. Each daughter nucleus has a characteristic bean-shaped outline, with the broader convex borders turned toward each other. The outline of the original interzonal substance is not distinct.

The direction of the furrow of cleavage corresponds exactly with the plane of the median axis of the adult organism. The two segments of the blastoderm correspond to the right and the left half of the adult organism respectively. The "median" or the "unpaired" structure as such, therefore, strictly speaking, has no existence in the squid. All parts are paired histologically, and derive their material from two bilateral sources. The median structure, the siphon, arises in that way, as is well known. Organs like the digestive tube, must also be considered as arising by the meeting of some descendant of cells derived from two halves of the original blastoderm such as I have been describing.

Fig. 19 represents a stage in which each of the two daughter nuclei of the previous figure is dividing. In the nature of the archoplasmic spindle and the appearance of the equatorial chromosomes, they do not offer any recognizable difference from those seen in a preceding stage. One important point to be noticed in this stage is the direction of the longitudinal axis of the caryokinetic figure in reference to the plane of the first cleavage furrow. The axis of the spindle does not run exactly in the same direction with the first cleavage furrow, but slightly diverges from it anteriorly. If the axes of the two spindles on both sides of the first cleavage furrow be prolonged both anteriorly and posteriorly, they will soon meet in the prolongation of the median plane of the blastoderm in the posterior part, while they will diverge more and more as they are prolonged in the anterior part.

This stage introduces us into the next one (Fig. 20), where the slight antero-posterior differentiations shown in the disposition of the caryokinetic figure in relation to a fixed plane in the

previous stage, becomes still more emphasized in the arrangement of the resulting nuclei. For the sake of convenience, I will call the segments in front of the second furrow of cleavage the anterior, and those behind it the posterior, segments of the blastoderm. The nuclei of the anterior two segments take different positions from those of the posterior ones. This is shown by the attitude they assume in reference to the median plane, as will be seen in the figure.

The antero-posterior differentiation of the blastoderm is made still more manifest in the succeeding stage, Fig. 21. The longitudinal axis of the caryokinetic figure in each of the posterior segments is almost parallel with the second furrow of cleavage, while the same axis in the anterior segment makes an angle of about 45° with it.

The antero-posterior differentiation indicated by the disposition of the caryokinetic figure in the previous stage is made still more apparent by the appearance of the cleavage furrows, Fig. 22. While the third furrows in the posterior segments run nearly parallel to the first and nearly at right angles to the second cleavage plane, those in the anterior segments take quite a different direction, as will be seen in the figure. They run parallel to, or even converge towards the median axis in the posterior half, while in the anterior half of the blastoderm they run away from the median plane.

In Fig. 23 the twelve segment stage of the blastoderm is shown. No two cells on one side of the blastoderm are alike, in size, in shape, or perhaps in their destiny. Just where the succeeding cleavage furrows come in, is indicated by the equatorial plane of the caryokinetic figure.

Fig. 24 represents the sixteen segment stage of the blastoderm. The bilateral arrangement of the segments is perfect. One important fact which will be discussed later may here be pointed out; viz. all the segments in front of the first cleavage furrow are in a more advanced stage of caryokinesis than those situated behind. This difference in the rate of growth in the anterior and posterior halves of the blastoderm is most pronounced in the behavior of four central cells, surrounded by twelve marginal cells. Two central segments which are situated anteriorly are considerably larger than the other two central segments situated behind. The former belong to the original

anterior half of the blastoderm, and the latter are derived from the original posterior segments.

The nuclei in the central segments are decidedly in different stages of growth. Those in the anterior segments are far more advanced than those in the posterior segments. The outlines of the anterior central segments present interesting points for study.

In the first place, we see that the shape of a given cell is determined by the condition of the environment; that is, it conforms to the space determined by the meeting of several other segments. The lateral horn on the outer border of each cell fits into the space between the heads of two marginal segments.

On the other hand, the same cell appears to me to offer another example of how the outline of a given cell may arise from another cause. For instance, the conical process at the anterior border of the same cell, penetrating into the substance of the marginal cell is produced by a different cause from that producing lateral horns already referred to. The anterior horn came into existence in connection with the production of the caryokinetic phenomena going inside of its cell boundary, and has some intimate connections with the archoplasmic sphere which lies nearest to it.

Fig. 25 represents the blastoderm at the twenty-two cell stage. The number of the marginal cells is twelve—the number we had in the preceding stage. The inner cells have been increased to ten from the four previously present. The history of the two pairs of inner cells of the previous stage (Fig. 24) is again repeated in the present stage. The two anterior segments which were further advanced than the posterior pair in Fig. 24, have nearly completed the division, and the posterior pair which were in a resting condition are in pretty well advanced stages of caryokinesis.

The right segment is, however, a little further advanced than the left segment. Two posterior marginal segments in direct contact with the two inner cells above described also show that the right segment is further advanced than the left one. The segments between second and fourth furrows of cleavage in the posterior half of the blastoderm, lying on the right and left side respectively of the two inner segments above described, show also difference in the degrees of division, the right half being in advance to that of the left.

Fig. 26 represents a blastoderm with 30 segments, with 12 inner cells with 18 marginal cells. Two of the inner cells have nearly completed division, but each has been counted as one. Most of the cells are in a resting condition, and the rate of growth is uniform on both sides of the blastoderm.

Fig. 27 represents the 32 cell stage, with 14 inner cells and 18 marginal cells — 16 cells on each side of the first or the second cleavage furrow.

If one, however, divide the blastoderm into front and behind by the furrow of the first cleavage, as is clearly indicated by the state of nuclear changes, we find an interesting contrast in the division of the inner and marginal segments. For while we find 10 marginal segments out of the entire 16 of the blastoderm in the front half alone, we find only 8 in the posterior half. In the quantity of the inner segments, however, the posterior half is ahead of the anterior; for the former contains 8, and the latter, only 6. Hence, while the anterior half is ahead of the posterior in the number of marginal cells, the latter is ahead of the former in the number of the inner cells; and hence the numerical superiority of the anterior half of the blastoderm is produced by cells of larger dimension than the cells in the posterior half, the anterior half of the blastoderm occupying a larger area than the posterior half. Besides the 6 inner cells in the anterior half occupy a larger area than the area covered by 8 inner cells in the posterior half, altogether making the development of the front half more conspicuous than that of the posterior half.

Fig. 28, Pl. XII, shows a 32 cell stage — 16 in front and 16 behind the second cleavage furrow ($2'-2$). The first cleavage furrow ($1'-1$) divides the blastoderm into two bilateral halves, of which the left side is more advanced than the right side, as the conditions of their nuclei clearly show. The numerical proportions of the marginal and the inner cells in the posterior and anterior halves are exactly the same as in Fig. 27 (Pl. XI), described already. The development of a pair of marginal segments in Fig. 28, the segments lying between $2'$ and 4, and 2 and 4 respectively, on each side of the blastoderm, is considerably different from the development of the corresponding segments in Fig. 27 or Fig. 26. In Fig. 28 the marginal segment ($4-2'$) does not reach the margin of the blastodisc at all,

as in (4-2') Fig. 27. The corresponding segment on the right side (4-2), Fig. 28, tapers gradually to a point as it comes nearer to the periphery of the disc, and does not expand as in (4-2) marginal segment in Fig. 27. In my former communication¹ I failed to trace the true homology of that diminutive marginal segment in Fig. 28 (4-2') and (4-2), and a certain confusion has resulted in the naming of the marginal cleavage furrows.

Fig. 29 shows the blastoderm in the same stage as Fig. 28. The arrangement of segments is pretty much like that in Fig. 27. An interesting point in this specimen is that there exists a pair of triangular areas in the anterior half of the blastoderm, where the cytoplasmic cleavage of the segments is not complete. I cannot tell whether this is due to the incomplete division or due to the refusion of the original segments once completely divided. At any rate, it is interesting to notice that the distribution of these areas of incomplete division is perfectly bilateral and can be traced originally to a pair of single segments in the anterior half of the blastoderm in the eight cell stage (Fig. 22, Pl. XI).

The faint cell-boundaries, such as are represented in the figure, were all that could be seen soon after the blastoderm was prepared fourteen months ago. The specimen is still in existence, but fusion of the segments is almost complete and the original faint boundary lines are hardly discernible. The area looks, in fact, like an unusually large marginal segment with four caryokinetic figures in it.

The remaining three stages, Figs. 30, 31, and 32, do not need any special comment. The condition of the nucleus in every cell has been faithfully represented, showing at a glance which segment is further advanced than the others.

Fig. 30 represents a stage with 60 segments—30 on each side of the median axis or the plane of the first cleavage furrow. Counted with reference to the plane of the second cleavage furrow, we find 32 segments in the front half of the original blastoderm, and 28 in the posterior, showing that growth in the anterior half is more vigorous than in the posterior. In other words, each of two segments in the anterior half of the blastoderm in the 4 cell stage, such as are shown in Figs.

¹ Johns Hopkins University Circulars. March, 1889.

20 and 21, has divided into 16 segments, while each segment in the posterior half has divided into 14. We must also bear in mind that the size of the inner cells in the posterior half is generally smaller than that of the anterior ones, showing that the original anterior half of the blastoderm is ahead of the posterior in the number of segments as well as in the actual bulk of the protoplasm. An examination of the caryokinetic figures will show that the segments on the left side are further advanced than those on the right side.

It is a difficult matter to decide of just how many segments a given stage of the blastoderm consists, when the division of cells is such a gradual process, and when there exist stages showing perfect transition from one condition to another. Such a stage is shown in Fig. 31. The number of segments differs according to the kind of criterion one takes in deciding what constitutes a completion of cell-division. One thing is, however, certain,—that the growth of the blastoderm, as indicated by the conditions of the nuclei in different parts, is not uniform. The right side of the figure is decidedly ahead of the left half, and the left quadrant in the anterior half of the blastoderm is decidedly behind the right quadrant in the same half.

Fig. 32 represents the blastoderm at the 116 cell stage. The left half of the blastoderm is in advance of the right half, although their line of demarcation does not exactly coincide with the plane of the first cleavage. The cells situated in the central portion of the blastoderm are backward in growth and division, as in all preceding cases.

The numbers around the marginal cells indicate the order of succession of cleavage furrows in the marginal zone alone, in this case, as well as in all of the preceding specimens we have described.

V.

By examining the stages of division more carefully, we find that in many cases the divisions of the different segments in the same ovum do not take place at the same time and with the same velocity. We further find a curious fact, that when one segment or a few segments on one side of the blastoderm show a tendency to vary in a certain particular direction, the corresponding segment or segments on the opposite side show the same tendency.

Figs. 24 and 27, Pl. XI, show two stages of cleavage. All the blastomeres in front of the second cleavage furrow (2'-2) are more advanced than those lying behind it, as shown by the stages of the caryokinetic figures. The nuclei in the posterior half of the blastoderm shown in Fig. 27 are all in the "resting" condition, while all of those in the anterior half have advanced as far as the equatorial plate stage. The same fact has been observed by Vialleton, in *Sepia*. If I represent the condition in a diagram, and reduce back all the segments to the original blastomeres from which they have sprung, it will be something like Fig. XVI.

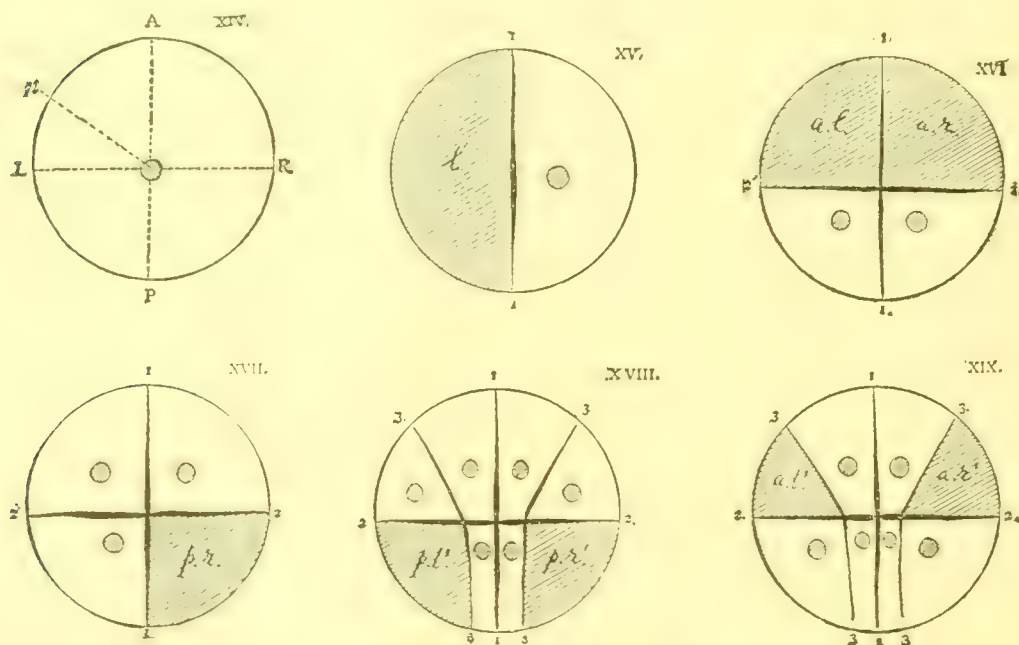


Fig. XIV. — The unsegmented blastoderm of the squid, showing the eccentric position of the nucleus *n*; A, anterior; P, posterior; L, left; R, right.

Fig. XV. — A diagram showing the one-sided development of the blastoderm. The segment *l* gave rise to all the segments in the left half of the blastoderm, such as are shown in Figs. 28, 30, 32.

Fig. XVI. — A diagram showing the unequal cleavage in the anterior and the posterior halves of the blastoderm. All the segments showing the more advanced stages in caryokinesis such as are shown in Figs. 24 and 27 were descended from the two anterior segments *a.l.* and *a.r.*

Fig. XVII. — A diagram showing precocious cleavage among the descendants of one segment, *p.r.*

Fig. XVIII. — A diagram showing the seats of abnormal (triple) caryokinesis, on the corresponding sides of the ovum, *p.l.*, *p.r.*

Fig. XIX. — A diagram showing the original segments, *a.l.*, *a.r.*, from which a pair of fused areas on both sides of the blastoderm (Fig. 29, Pl. XII) were descended.

The first furrow of cleavage is indicated by (1'-1); and the second furrow by (2'-2); *a.l.*, *a.r.* represent the anterior blastomeres. All the cleavage segments in front of the line (2'-2) in Figs. 24 and 27, showing the more advanced condition of caryokinesis than those lying behind, were descended from these two blastomeres *a.l.* and *a.r.*, as represented in the diagram (XVI); and all those segments showing the backward conditions in their nuclear changes were derived from the two segments which lie behind them.

Fig. 25, Pl. XI, shows another interesting variation. All the four segments, which descended from one quadrant (*p.r.*, Fig. XVII) are further advanced than their homologues on the opposite half, which have descended from the corresponding quadrants on the opposite half.

Figs. 28, 30, 31, 32, were all taken from the eggs of one and the same squid. As the examination of caryokinetic figures in each segment of the blastoderm will show, those segments lying on the one side of the first cleavage furrow are more advanced than those on the other, making exceptions of those few segments which are found along the posterior half of the median axis.

I cannot well describe the extremely slight differences shown by different blastomeres in different parts of the blastoderm. A glance over the figures and the comparison of different segments will show them far more clearly than pages of verbal description.

As I have stated already, Fig. 31 was taken from the same lot of eggs as Figs. 28, 30, and 32. But it is the right side of the blastoderm which shows a more advanced condition, and not the left side, as in the others. I am inclined to believe now that in this case, the blastoderm was mounted with the wrong side up, and what appears as the right side in the figure belongs really to the left side of the animal. If such was the case, the left-handed variation, as shown in the series of four stages, Figs. 28, 30, 31, and 33, becomes interesting, since all the eggs come from one and the same animal. It is probable that this tendency to vary in the same direction may be due to the same hereditary characteristics inherited from the parent organism.

Fig. 29 is an interesting example of what may be designated as the analogous variation of both sides of the germ. Four segments on each side of the blastoderm show a curious phenom-

enon of fusion. It may have been an imperfect division at first, however. At any rate, the behavior of these four corresponding segments on each side of the median axis is interesting. The specimen as it now stands has lost all the faint cleavage lines which existed at first, and the four blastomeres have completely fused with each other. The origin of this mass of fused segments can be traced to a single blastomere at the eight cell stage. In the diagram, Fig. XIX, the segments *a.l'* and *a.r'* on both sides of the first cleavage furrow (I'-I) represent the original segments which gave rise to the four imperfectly divided segments described above.

Another curious example which I have met is one in which the abnormal caryokinesis in one segment on one side of the body is shared by the corresponding segment on the other (*p.l'* and *p.r'*, Fig. XVIII). In both segments mentioned above, the nuclei were seen in the state of being divided into three nuclei, as indicated by the triasters.

The fact that the groups of cells which vary simultaneously on both sides of the bilateral ovum can be traced back to a single segment in an earlier stage appears to me to be a matter of considerable importance. Although I was unable to trace the whole history of such segment or segments, it is probable they give rise to or constitute a corresponding part of some future bilateral structure. Another fact, namely, the unusual mode of caryokinesis occurring in the corresponding segments of the bilateral blastoderm when all other cells in the body are dividing normally, is a point worthy of consideration.

And finally, we must bear in mind that the precocious cleavage may occur in the descendants of a single blastomere alone and not in pairs, as in Fig. 24.

This phenomenon of *unequal growth of different parts*, as I may call it in an empirical way, has been observed by several naturalists.

Brooks¹ figured unequal cleavage in the bilateral blastoderm of *Batrachus*.

His² developed this fact of unequal growth of different parts into a principle, although his principle more especially refers

¹ *Alternation of Periods of Rest with Periods of Activity in the Segmenting Eggs of Vertebrates*. Studies from Biol. Laborat., Johns Hopkins Univ., Vol. II, 1882.

² *Unsere Korperform*, etc., 1874.

to a much later stage of embryonic development with special application to the facts of organogeny. Newport¹ noticed long ago, in the frog, that "about two hours after the completion of the crucial cleft [the second furrow of cleavage] a new series of changes is set up in the egg. The clefts no longer include the whole circumference of the egg, but are confined to the splitting of the larger into smaller pieces, after a binary plan; and this process does not begin at once over the whole surface, but appears first in a given spot, and then pursues a definite course; thus each of the two pieces seen from above on one side (behind) of the crucial cleft becomes subdivided, producing four segments on one side of that line, whilst there are only two on the other. When this subdivision is nearly completed, and not till then, a corresponding change takes place in the two segments on the other side (in front) of the sulcus."

What are the effective causes which produce these differences in different parts of one and the same blastoderm?

Unless we study different forms more extensively, discussion in this field is not likely to be of any particular value. The following remarks are therefore only provisional ones, with special reference to the results of my Cephalopod study.

Growth of any two given parts in an organism may be similar or different, according as the inherent nature of the material which constitutes the given parts are similar or different. Two parts fundamentally alike, on the other hand, may grow at different times and at different rates, according to the conditions of environment which affect them. In other words, unequal growth may take place in two parts intrinsically similar, one of the parts, however, being more favorably situated than the other.

By which of these categories of causes may the unequal growths we have seen in the blastoderms of the squid be accounted for? To characterize this whole series of variation simply as pathological is not an explanation. On the other hand, it has been suggested that the unequal growth such as is manifested in the alternate cleavage of the squid ovum may be explained as due solely to the influence of external conditions. I am, however, disposed to believe that external conditions have nothing to do

¹ *Researches on the Impregnation of the Ovum in the Amphibia; and on the Early Stages of Development of the Embryo.* 3d Series. Philosophical Transactions, 1854, p. 241.

with this particular case. My reasons are as follows: I have shown that the area which is the seat of a particular variation either in the behavior of the nucleus or of the cytoplasm, such as the peculiar fusion or the incomplete division of the cytoplasm, or in the velocity of the caryokinetic division, can always be traced to one single segment in a still earlier stage. In other words, when an area consisting of a small number of cells shows a peculiar modification, that area can always be traced to a single cell in a still earlier stage, from which it has descended. When analogous phenomena of variation occur on both sides of the bilateral axis of the blastoderm, the area where such phenomena occur can be traced to one corresponding cell on each side of the axis, which came into existence at the same time on both sides. Or when the variation occurs only on one side, or at one spot, this area of variability is made up of the descendants of a single cell.

It is difficult to suppose that the environment affects one quadrant of the blastoderm at the four cell stage different from another; or how the external conditions can affect the corresponding segments on each side of the blastoderm and divide their nuclei with three asters, when all the rest of the nuclei are divided with two, it is difficult to imagine.

The cause of unequal cleavage in the various cases we have examined appears to me to be an internal one, due to the peculiarities of the particular protoplasmic structure which composes the segment or segments. When, therefore, the right and left halves of the bilateral blastoderms show a difference of velocity in their cleavage, I believe it is due to the slight qualitative inequalities induced by the first division,—inequalities which appear more and more exaggerated as the cleavage process advances.

These facts seem to point to two conclusions:—

1) That the earlier cleavage processes are more fundamental, and, from the morphological standpoint, more significant than the later ones.

2) That, as I have already mentioned, since the eggs from the same animal show similar variations in cleavage, such a tendency to vary may become hereditary. This conclusion is, however, a provisional one.

EXPLANATION OF PLATE IX.

Loligo Pealei.

All the figures in this plate have been magnified 45 diameters.

FIG. 1. Unsegmented ovum, with three polar globules, one of which has not been made distinct by the engraver. The whitish cap at the pointed pole of the egg is the germ-disc.

FIGS. 2-8. Different stages of cleavage. The eggs have been slightly tipped over so as to show the cleavage field better.

FIGS. 9, 10, 11. Side views of more advanced stages of cleavage, showing the relation of the marginal elongated cells to the yolk-mass and to the central smaller cells.

FIGS. 12, 13, 14. Views from the cleavage-poles.

FIG. 15. An abnormal specimen, with the germ-protoplasm at the side of the ovum, and not at the top as is usual. Cleavage is, however, normal.



EXPLANATION OF PLATE X.

Loligo Pealei.

All the figures in this plate have been magnified 90 diameters. Polar globules have not been drawn in the figures.

FIG. 16. Unsegmented stage of the germ-disc. There are considerable variations in the size of the germ-disc in different ova. This is an unusually large one. Notice that the anterior half of the germ-disc is larger than the posterior half.

FIG. 17. A germ-disc at the midst of the first cleavage.

FIG. 18. A germ-disc at the completion of the first cleavage furrow.

FIG. 19. A germ-disc at the midst of the second cleavage.

FIG. 20. A germ-disc at the conclusion of the second cleavage. Gaping of the first cleavage furrow at the anterior and posterior margin of the blastoderm in this figure as well as in the next, is produced by the pressure of the cover-glass on the convex germ-disc.

FIG. 21. A germ-disc at the beginning of the third cleavage. Notice the difference in the directions of cleavage in the anterior and the posterior half of the disc, as indicated by the directions of the caryokinetic axes.

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EXPLANATION OF PLATE XI.

Loligo Pealei.

All figures in this plate have been magnified 65 diameters.

FIG. 22. A blastoderm consisting of 8 cells, 4 in front, and 4 behind the second furrow of cleavage. All the cells in front are larger than those behind.

FIG. 23. A blastoderm consisting of 12 cells.

FIG. 24. A blastoderm consisting of 18 cells. All the segments in front of the second furrow of cleavage are more advanced than those behind it.

FIG. 25. A blastoderm consisting of 22 cells.

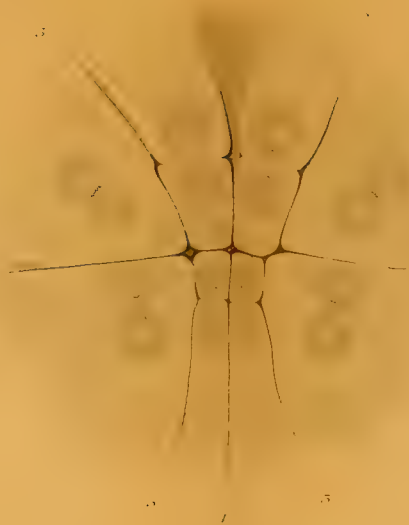
FIG. 26. A blastoderm which has just entered into the 32-cell stage. A wide cleft in the left side of the blastoderm, 2', corresponding to the second furrow of cleavage, was produced by the separation of the adjacent segment due to the pressure by cover-glass.

FIG. 27. A blastoderm consisting of 32 segments, with all the segments in the front half of the blastoderm in the midst of division, while the nuclei in the posterior half are in a "resting" condition. A group of 6 inner cells, and a pair of marginal cells along the first cleavage furrow, in the posterior half of the blastoderm, present the characteristic appearance of this stage. As the later stages will show, these groups of cells make the slowest growth in the whole blastoderm.

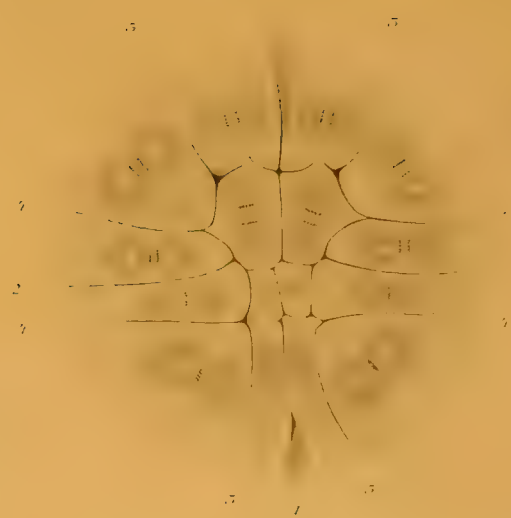
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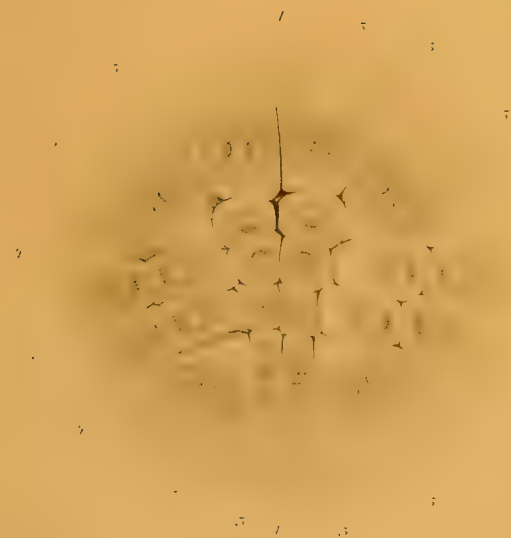
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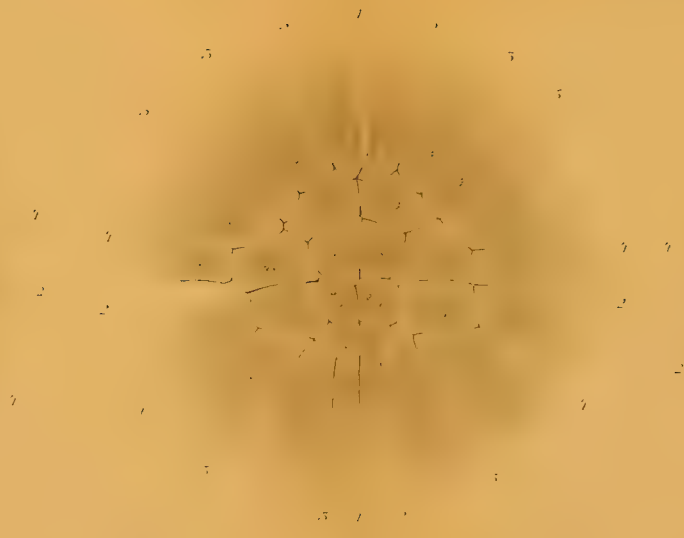
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EXPLANATION OF PLATE XII.

Loligo Pealei.

All the figures in this plate have been magnified 65 diameters.

FIG. 28. A blastoderm with 32 cells. The left half is ahead of the right half in the progress of division.

FIG. 29. A blastoderm consisting of 32 cells. This specimen shows a pair of peculiarly fused areas which are symmetrically arranged on both sides of the median furrow of cleavage.

FIG. 30. A blastoderm consisting of 60 cells. The left-hand side of the blastoderm is further advanced than the right side.

FIG. 31. A blastoderm consisting of 90 cells. In this specimen, the right-hand side is further advanced than the left half.

FIG. 32. A blastoderm consisting of 116 cells. The left half ahead of the right.

All the specimens represented in Figs. 28, 30, 31, and 32 have been obtained from the eggs of the same squid.





CONTRIBUTIONS ON THE MORPHOLOGY OF THE ACTINOZOA.

II. ON THE DEVELOPMENT OF THE HEXACTINIÆ.

J. PLAYFAIR McMURRICH.

FOR the past few years I have been endeavoring, as occasion offered, to obtain the material necessary for the study of the development of some Hexactinian, but my efforts have been only partially successful. In 1887 I obtained at Nassau, Bahama Islands, W.I., a few embryos of two different Actiniaria, — *Aulactinia stelloides*, McM. and *Rhodactis Sancti Thomæ* (Duch. and Mich.). Both these forms, however, retain the embryos in the interior of the body until the mesenteries have formed, the embryos from *Aulactinia* possessing, when extruded, from eight to twelve perfect mesenteries, while those from *Rhodactis* are extruded somewhat earlier, while they are furnished with only two or four perfect mesenteries. These forms, therefore, agree, as regards the retention of the embryos, with the majority of forms whose embryology has been studied.

There are some forms, however, in which the ova are extruded unfertilized. *Adamsia parasitica* is one of these according to Kowalewsky ('75), and I have found that *Metridium marginatum*, so abundant on the New England coast, does so likewise. During the summer of 1889, aided by the generous enthusiasm and energy of two assistants, I was able through the facilities offered by the Marine Biological Laboratory at Woods Holl, Mass., to collect and keep large numbers of this Actinian in aquaria, and so obtained some ova which were artificially fertilized by sperm extruded by other individuals at the same time. Unfortunately spermatozoa were not always obtainable when required, and consequently I was obliged to allow a large number of ova, obtained at various times, to decay, and was only successful in rearing embryos in a very limited number of cases. There are consequently many gaps in my observations, which might readily have been filled in by the

study of more abundant material. Those ova which were fertilized I did not succeed in rearing up to the time of formation of the mesenteries, owing probably to ignorance of the proper food which they require at this period. I had hoped to be more successful during the summer just past, but my endeavors to obtain ova were entirely fruitless, though spermatozoa were plentiful.

1. THE SEGMENTATION AND FORMATION OF THE GERM-LAYERS IN METRIDIUM.

In his classic monograph on the development of the Actinians Lacaze-Duthiers ('72) advances the suggestion that these forms are hermaphrodite. Later observers have not succeeded in confirming this opinion in so far as the Hexactinians are concerned, though the coexistence of ova and spermatozoa has been found in certain Zoanthææ and Cerianthææ. I have never found in *Metridium*, or in any of the numerous other Hexactiniæ I have examined, any trace of hermaphroditism, and believe that it may be accepted as a rule that the Hexactinians are bi-sexual. Dichogamy may possibly occur, but the evidence seems strongly against even this.

The immature ova of *Metridium* lie closely packed together in the mesogloea of the gonophoric mesenteries, mutual pressure compelling them to assume irregular shapes. When set free by teasing, the younger ova are almost spherical, while the older ones (Pl. XIII, Fig. 1) are somewhat irregular, the majority possessing a more or less elongated process extending out from the circumference at one point, sometimes in the neighborhood of the nucleus, sometimes distant from it. The nucleus is large, with a single large nucleolus, and is always situated eccentrically, lying as a rule nearest that pole of the ovum which is adjacent to the free surface of the mesentery. The peculiar filamental apparatus which the Hertwigs have described in connection with the immature ova of *Adamsia parasitica* ('79) and other forms ('82), I have never observed in *Metridium*.

The mature eggs are perfectly spherical, pinkish in color, and opaque with small granules of food-yolk. They measure 0.124 mm. to 0.159 mm. in diameter, and each ovum is surrounded by a delicate membrane in which no perforations are visible. No nucleus can be detected in the fresh ova after their extru-

sion, though they are evidently thoroughly mature, and ready for fertilization, after which segmentation begins without any intermediate formation of polar globules. It seems almost certain that these bodies are formed before the extrusion of the ova, possibly while they are still in the mesenteries. I have endeavored in vain to observe their formation, but do not attribute my want of success to their non-existence. Haddon ('89) has figured (Pl. XXXVI, Fig. 10) a condition which he suggests as representing the formation of a polar globule while the ovum is still in the mesentery, but I cannot consider the formation of polar globules before the setting free of the ovum, to be proved by what he has figured, there being no evidence, such as the existence of karyokinesis, to show that the body being extruded from the ovum really represents a polar globule. It seems more probable that the figure represents an artefact.

The first indications of segmentation occurred in the ova of *Metridium* about three-quarters of an hour after adding the sperm to the water containing the ova, and consisted in the formation of two slight elevations at one pole of the egg (Fig. 2), reminding one very much of what occurs in the ova of Ctenophores. A slight groove appears later between the elevations, and, gradually deepening (Fig. 3), finally cuts the ovum into two spherules. I did not observe the formation of the elevations in all cases, but it seems certain that the segmentation furrow always starts at one pole of the egg in the typical Cœlenterate manner,—a method of segmentation, by the way, which does not seem by any means to be confined to that group, but to be of more widespread occurrence.

The two spherules are usually slightly unequal in size (Fig. 4), but the amount of the inequality may vary considerably, being well marked in some cases, but hardly noticeable in others.

When the segmentation furrow is completed, the spherules are rounded, and in very slight contact with each other. Soon, however, the contiguous faces flatten down (Fig. 4), and a refusion of the spherules occurs. This phenomenon is very marked in the four-celled stage. I have seen the spherules in this stage become reduced by refusion to two, which, on their part, underwent refusion, so that there was a return to an apparently unsegmented ovum. I was not able, however, to watch the further development of this egg, so cannot say that it was

perfectly normal, but it is certain that the refusion phenomena are well marked.

At the close of the two-celled stage the ovum is slightly oval, and shows no external trace of having consisted of two distinct spherules. It then divides at once into four spherules, the cleavage furrows arising at the exterior and passing towards the centre, the segmentation belonging to that variety which Metschnikoff distinguishes as centripetal ('86). Two of the spherules are usually smaller than the other two, and two lie upon a different plane than the other two (Fig. 5). How this is brought about I cannot say; it may be due either to the nuclei of the original spherules dividing obliquely to the plane which originally separated them, or else to the nucleus of one of the original spherules dividing in a plane at right angles to that in which the other divides, as Ludwig has described in *Asterina* ('82).

Subsequent divisions lead to the formation of eight (Fig. 6), sixteen, thirty-two, and sixty-four cells, although occasional irregularities were seen, such as stages in which there were seven or eleven cells. The irregularity of the spherules evident in the earliest stages becomes reduced as development progresses, so that it becomes impossible to orient the embryos in later stages, and so determine the relation of the axes of the ovum and embryo. Inequalities are to be seen in the cells of young blastulas (Fig. 7), but they are irregular, and do not serve to mark out a pole of the embryo. The result of segmentation is the formation of a hollow blastula, with walls composed of elongated columnar cells richly packed with granules of food-yolk, with a few clear vacuoles, and with their small nuclei situated peripherally. This blastula is somewhat pyriform in shape (Fig. 8), and is ciliated, swimming about at the bottom of the vessel in which it is contained, the narrower end being anterior, and progression being accompanied by rotation about the long axis. The cilia cover the entire body, and are equal in length except at the pole which is anterior in swimming, where there is a tuft of longer cilia, similar to what has been found in other Actinian larvæ. In sections of embryos in this stage the interior appeared to be quite empty, but in others again (Fig. 11) it was filled with what seemed to be a coagulable fluid, in which were scattered granules of food-yolk evidently derived from the partial

disintegration of certain of the blastula cells. As will be seen later, this disintegration is a normal occurrence, but the time when it makes its appearance seems to vary in different cases.

Viewed externally, the larva seems to persist in this condition for some time (Fig. 9), but sections show that very important changes are going on during this apparent rest. These changes consist of the formation of the endoderm by delamination. I was not able to observe the nuclear phenomena accompanying this process, but there seems little room for doubt that it really is a delamination. It is multipolar in its distribution, and cuts off the peripheral third from the inner two-thirds of each cell (Fig. 12). The outer cells so formed become somewhat spherical, are somewhat granular, and show a distinct nucleus. A peculiar feature which characterizes them, and which seems to be invariably present after delamination is completed, is a clear vacuole, larger than the nucleus, occupying the central end of each cell; somewhat similar vacuoles are figured by E. B. Wilson ('83) in *Renilla*, but what may be their significance is not apparent. The inner or endoderm cells are much larger than those belonging to the ectoderm, and like these are granular with food-yolk, and here and there show a clear vacuole similar to those of the ectoderm cells, but having no definite position in the cell. One peculiar feature of the endoderm cells is the difficulty which exists in observing their nuclei. In specimens in which the delamination is just beginning, only a few cells having separated into the endodermal and ectodermal moities, bodies which stain somewhat more deeply, and are as a rule somewhat larger than the yolk-granules, can be observed scattered about at different levels in the inner portions of the wall of the blastula. Only one of these bodies is to be seen in each cell, there being in addition, of course, the nucleus near the periphery, which will eventually belong to the ectodermal moiety. They do not stain nearly as deeply as the peripheral nuclei, but nevertheless I am inclined to believe them to be the nuclei of the endodermal cells, it being evident from the presence of the large vacuole at the junction of the outer and middle thirds of the cells in which they occur that they are quite ready for division (Fig. 11). When the delamination is complete, however, these bodies cannot be satisfactorily made out, and the endoderm cells are apparently without nuclei,

though it seems more probable that they are actually present, though undiscoverable. H. V. Wilson ('88), it is to be noticed, found the same difficulty in observing the nuclei of the delaminated endodermal cells of *Manicina*.

As already remarked, the cavity of the blastula becomes more or less filled by a mass of yolk-spherules. Occasionally the appearance of these spherules may occur quite early, but at other times, and perhaps more normally, it is delayed until the beginning of delamination. The granules come from the breaking down more or less completely of the endoderm cells. That they have this origin is, I think, clearly shown in Figs. 11 and 12. In the former the disintegration seems to have affected some cells to a very great extent, but in the latter it seems for the most part to be only the central ends of certain cells that are affected. The central mass is certainly not cellular, the cell outline having become obscured, as in certain other Actinozoa, but is composed simply and solely of yolk-granules, sometimes imbedded in a homogeneous coagulable matrix. In some cases where the central matter made its appearance during the blastula stage, the inner ends of certain cells seemed to fall off into the central cavity, and then to undergo disintegration, but in no case could there be said to be actual cellular elements in the central mass. This is a point of considerable importance, having very decided bearing upon what has been described for other Actinians, and allowing of inferences as to the actual occurrence in these superficially studied cases.

At the conclusion of the delamination a slight depression is to be observed at the posterior pole of the larva (Fig. 9), and soon after this breaks through, a communication of the interior cavity with the exterior being thus established. The endodermal cells have by this time arranged themselves in a definite layer, separated by a slight space from the ectoderm, and the larva has the appearance of a typical invaginate gastrula (Fig. 10). In fact, before making my sections, I was persuaded that the formation of the germ-layers was by invagination. It is not easy to observe in optical sections the endoderm cells soon after their delamination, and larvæ with the slight depression at the posterior end appear to be single-layered (Fig. 9), the depression seeming to be the commencement of an invagination. The fact that I was not able to observe any intermediate stages between

embryos with the depression and the fully formed gastrula surprised me somewhat, but still the appearances presented by optical sections seemed so clear, and the gastrula so similar to an invaginate gastrula, that I believed that I had missed or overlooked the intermediate stages. Sections, however, explained the matter at once, and demonstrated that instead of with invagination we have to do with delamination. I make special mention of this mistake, since, as I shall shortly endeavor to show, other observers have probably fallen into the same error.

As to what becomes of the yolk-spherules occupying the central cavity, I have no evidence. It is possible that they may be absorbed by the endoderm cells as soon as they have reached their final differentiation, or they may pass to the exterior through the blastopore and be lost to the larva.

Beyond this stage I was unable to rear the embryos. A few Actinozoan larvæ obtained by skimming are almost certainly those of *Metridium*, but I prefer to leave them undescribed for the present, partly on account of the uncertainty of the identification and partly because they belong to later stages which may be more satisfactorily studied in the larger embryos of *Rhodactis* and *Aulactinia*.

Between the latest stage of *Metridium* and the earliest which I possess of *Rhodactis*, there is an hiatus, during which there occurs the formation of a very important and characteristic structure, the stomatodæum. Still, from what occurs in other forms, we may readily imagine how its formation takes place in *Metridium*. *Cerianthus* apparently has a stage almost if not quite identical with the last stage of *Metridium* described, and in this form the lips of the "gastrula" mouth bend in to form the two-layered stomatodæum. It seems probable that the same thing occurs in *Metridium*.

A comparison of the methods of formation of the germ-layers in the Actinozoa, so far as they are known, is of considerable interest. Our knowledge is derived from the study of only a relatively small number of forms; nevertheless, it suggests some important ideas. The amount of food-yolk seems to have a very important influence on the details of the development, but the general mode of formation of the germ-layers is the same in the majority of forms. Amongst the Alcyonaria we have accounts

of the early development of at least four different forms. In *Alcyonium digitatum*, according to Kowalewsky ('73), the finely granular protoplasm separates in the form of spherules from a central coarsely granular mass, which thus becomes enclosed by protoplasmic spherules, and later on divides up into a number of cells. In *Clavularia* (Kowalewsky and Marion, '83) the yolk does not exert so great an opposing influence to the division forces, and we see the planes extending much further towards the centre of the egg than in *Alcyonium*, although there is at first a small portion of yolk which does not share in the division. It gradually disappears, however, with the later divisions, and an embryo similar to that of *Alcyonium* is formed. In *Renilla* (E. B. Wilson, '83) the phenomenon is essentially the same as in *Clavularia*, but in *Sympodium* (Kowalewsky and Marion, '83) the segmentation seems to be total. The early phases of segmentation have been thoroughly worked out only in *Renilla*, and we know that in it considerable variation may occur in the manner of division, the ovum sometimes dividing at once into eight, sixteen, or even thirty-two cells, besides showing other peculiarities. This did not depend on the sudden division of the nucleus into such a number of parts, but the nuclear division went on, no doubt, in the usual manner, the cytoplasmic division being inhibited to a greater or less extent by the amount of food-yolk present. In the segmentation of *Alcyonium* the same process is apparently carried still farther.

In all these forms, and in *Gorgonia Cavolini* described by Von Koch ('87), the segmentation leads to the formation of a solid structure consisting of an external layer of cells (ectoderm) surrounding a solid central mass of cells. This is the organism for which Metschnikoff ('86) has proposed the term *Parenchymella* or *Phagocytella*,—rather cumbersome names, which may perhaps be more conveniently replaced by *Sterrella*. In only one of the forms studied have we definite evidence as to the manner of formation of the central mass, and that is in *Renilla*, in which Wilson has shown that a *Sterroblastula* (Goette) results from the segmentation, and that the inner ends of its cells delaminate off to form the central solid mass. The similarity of the *Sterrellas* of the other forms to that of *Renilla* seems to indicate that the same process of delamination occurs in them likewise. It certainly cannot be a process of invagination which gives rise

to the Sterrula, and there are physical difficulties in the way of immigration.

There has been described, however, among the Alcyonarians a single case of invagination following the formation of an archiblastula. Haeckel ('75) states that he has found such an occurrence in *Monoxenia*. I have not been able to consult his original account ('75 a) of this phenomenon, and cannot accordingly determine the value of the statement; but in view of what is known to occur in other Alcyonaria, and since he also states that he has found the same thing in an Actinian, — which, as I shall later endeavor to show, seems doubtful, — I think it is not safe to rely on the accuracy of the statement until it is confirmed by further observations.

Up to the present, we know nothing as to the formation of the germ-layers in the Edwardsiæ, the recent representatives of the ancestors of the Hexactiniæ; but the Alcyonarians must be regarded as standing with the Edwardsiæ at the bottom of the Actinozoan stem, since the two groups agree in the number of their mesenteries, — a feature of fundamental importance in the present state of our ideas on the subject of the evolution of the Actinozoa, the difference in the mode of arrangement of the longitudinal muscles being of secondary value. The occurrence of a Sterrula, and its formation by delamination in this somewhat primitive group of Actinozoa, is accordingly of importance as affording a basis whereon to build an explanation of what is found in the Hexactiniæ.

The most definite statements made hitherto as to the early development of the Hexactiniæ are by H. V. Wilson ('88), for *Manicina*. Segmentation results in the formation of a hollow blastula, and by the delamination of the blastula cells, the blastocœl becomes filled up with a mass of yolk-bearing cells whose outlines and nuclei cannot readily be made out. The embryo is then a Sterrula, and agrees in the manner of its development with the Alcyonaria. Jourdan's description ('80) of the development of *Balanophyllia* does not extend to stages sufficiently early to enable one to state what the method of formation of the germ-layers is; but it is probable that it is closely similar to what occurs in *Manicina*, and a Sterrula (see his Fig. 126, Pl. XVII) is the result.

In *A. parasitica* (*Adamsia Rondeletii* of Andres), Kowalew-

sky ('75) found the method to be as follows: "This phenomenon (the segmentation) is quite regular, — *i.e.* the egg divides into two spherules, then into four, and so on. There results from this segmentation a mass of cells without the formation of that central cavity which is termed the segmentation cavity. After the segmentation is completed, the embryo covers itself with vibratile cilia, and begins to swim." A Sterrula is consequently formed in this case likewise, though from the description quoted it is impossible to state the exact manner in which it arises.

Before proceeding to compare these forms with *Metridium*, it will be necessary to mention certain cases in which an invaginate gastrula has been stated to occur. These are a form related to *A. mesembryanthemum* and *Cerianthus*, according to Kowalewsky ('75 and '73), and *A. equina*, according to Jourdan ('80). I regret exceedingly that Kowalewsky's important paper is inaccessible to me, and I have been obliged to rely on the abstract given in Hoffman and Schwalbe's Jahresbericht, and on the translation of a portion of the original paper by Giard (Kowalewsky, '75). As for the figures, I have been able to see only those reproduced by Mark ('84), in Pls. XI and XII of the Selections from Embryological Monographs. Fig. 27 of Pl. XI, representing the gastrula of *A. mesembryanthemum* (?), is very similar to that given by Jourdan from *A. equina*, and it certainly looks like an early stage of invagination. Nevertheless, it seems to me, in view of what I have described for *Metridium*, that there is a very great chance that both Kowalewsky and Jourdan have been mistaken in their interpretation of what actually occurs. I have found in *Metridium* what I considered malformations, which approach very closely to what these authors describe and figure. I find in my notes the following statements: "In many (*i.e.* swimming blastulas) a depression could be observed upon one side, giving rise to a slight projection into the blastocœl. The invagination (*i.e.* depression) appears to occur mostly at the side. Never saw one at end." I have drawings of optical sections of this which present an appearance not very unlike what Kowalewsky and Jourdan have figured. It must be noticed, too, that neither of these authors were able to trace directly one of these stages into the fully formed gastrula stage. Jourdan gives as the suc-

ceeding stage a form in which the ectoderm and endoderm are well differentiated histologically, the endoderm cavity is filled up with food-yolk, and the stomatodæum has already formed. There is evidently a great hiatus between the two stages. Kowalewsky's description ('75), likewise, leads one to infer that he observed only isolated stages, and did not actually witness the conversion of the early invagination stage (Mark, '84, Pl. XI, Fig. 27) into the fully formed gastrula (Fig. 28), which, it is to be noticed, is exactly similar to my figure of the gastrula of *Metridium* (Pl. XIII, Fig. 10).

These facts, taken into consideration with the difficulty of observing the delamination by optical sections, render it not improbable that the interpretation of the figures referred to is erroneous, and that what really occurs is what I have described for *Metridium*, and there is a further point which renders this probability almost a certainty. Jourdan's Fig. 117 shows the endodermic cavity to be filled with food-yolk, which is not present in the stage represented in Fig. 116. Whence does this food-yolk come? Kowalewsky's figures of the gastrula of *Cerianthus* (Mark, '84, Pl. XII, Figs. 2 and 3) show essentially the same thing, though the amount of food-yolk present in the endodermal cavity is much smaller. Kowalewsky believes ('73) that the food-yolk is secreted by the endoderm cells after invagination, and is later on reabsorbed by them — a very remarkable proceeding. In the gastrula of *Metridium* food-yolk is to be found in the endoderm cavity, since it occurs in the blastocœl, and I think it exceedingly probable that future observations will show that, instead of invagination occurring in these forms, the process of endoderm formation is essentially what I have described above for *Metridium*. The cases of supposed gastrulation by invagination which have been described for the Hexactiniæ must be denoted, along with Haeckel's account of *Monoxenia*, as *not authenticated*.

In all the Actinozoa, then, concerning which we have reliable information, the endoderm is formed by delamination. It remains now to be considered whether what we find in *Metridium* or in *Renilla* is the more primitive. This inquiry leads to another; namely, *Was delamination the primitive method of endoderm formation, or is it a secondary modification of a still earlier phenomenon?*

An affirmative answer to the first part of this question formed the basis of Lankester's planula theory ('77), while the second part of the interrogation is considered a correct statement of affairs by the supporters of the Gastræa theory, invagination being according to them the original phenomenon. It is difficult to understand how one process could have been converted into the other, as is required by either theory. Lankester's theory, too, finds little support from the facts of development in the higher forms, whereas the Gastræa theory is strengthened by them. On the other hand, the Gastræa theory weakens when we come to study the developmental phenomena of the lower Metazoa.

I have already shown the improbability of the occurrence of an invaginate gastrula in the Actinozoa. In the Scyphomedusæ, it is certain apparently that it occurs in *Pelagia* and *Nausithoe*, but these are certainly exceptions. Goette ('87) has shown that a gastrula-like structure may be produced in *Aurelia aurita* without invagination, by the hollowing out of a Sterrula; and I have found this same structure to be formed by the immigration of certain of the blastula cells in *Cyanea artica*. *Lucernaria*, which is probably a very primitive Scyphomedusan, likewise forms a Sterrula (Kowalewsky, '84), and, so far as is known, an invaginate gastrula occurs in the Scyphomedusæ only in two isolated cases. The formation of a Sterrula is to be regarded as typical for the Scyphomedusæ.

In the Hydrozoa not a single case of invagination is known! See then what a showing we have among the Cnidaria, only two authentic cases of invagination for the formation of the endoderm are known!

How then is the endoderm formed in this group? Delamination occurs in the Actinozoa and in certain Hydrozoa, but there is a process back of this, from which it has been derived: I mean immigration. The most suggestive and admirable work of Metschnikoff ('86), has placed the occurrence of this process beyond a doubt; and that author has been led to regard the Parenchymella (Sterrula) as an ancestral form of the Coelenterates, from which, as he shows, a gastrula by invagination might readily be derived. With this opinion I desire to express my full agreement, and I have elsewhere ('91) endeavored to extend the idea somewhat. It will be unnecessary, therefore,

to enter into a lengthened discussion of the various subordinate and, so far as the essentials of the theory are concerned, unimportant details, which I have there discussed.

There is one point, however, which bears directly upon the question before us at present, and that is the origin of delamination from immigration. Metschnikoff's explanation of this is not, I think, quite satisfactory. He supposes, in fact, that the two phenomena are contemporaneous, and that both existed in the ancestral Metazoa, the endoderm being formed by "mixed delamination"; *i.e.* partly by true delamination and partly by immigration. Where, therefore, in the embryos of higher forms we find immigration alone occurring, there has been a secondary specialization, the delamination having been suppressed; similarly with forms in which delamination alone occurs. It seems to me, however, that, taking into consideration the occurrence of immigration alone in such forms as *Volvox* and *Protospongia*, the absence of delamination so far as is known among the Sponges, and its comparatively rare occurrence among the Hydrozoa, since the formation of a solid morula instead of a blastula, such as occurs for instance in *Hydractinia*, is to be regarded as precocious immigration rather than precocious delamination,—taking these facts into consideration, it would seem that immigration was a more primitive phenomenon than delamination.

I think a clew to the transformation of the one process into the other is to be found among the Actinozoa, in which, as I have shown, we have variations from such a delamination as occurs in *Alcyonium*, to what we find in *Metridium*. In *Renilla* the protoplasm is so weighted down that the completion of the division plane is difficult. The yolk is segregated towards the central part of the ovum, the periphery being more purely protoplasmic, and in the later stages the nuclei of the cells are in the peripheral portion. By segmentation a Sterroblastula is formed. There are, consequently, physical difficulties in the way of immigration, and, in addition, the food-yolk renders each cell as a whole inert, and deprives it of the energy necessary for immigration. The peripheral protoplasm, however, is unhampered by yolk, and cuts loose from the central yolk-containing portion, a differentiation of the Sterroblastula into a Sterrula thus occurring by delamination. There seems

to be here a difficulty, in the fact that immigration is not accompanied by a division of the cell. Immigration, however, must necessitate a considerable expenditure of energy, by the cell in general, which energy, it seems quite conceivable, might be turned, when immigration became unnecessary or impossible, to the production of a division.

Supposing the delamination to have arisen in this way in the lower Actinozoa, it is probable that on a subsequent loss of food-yolk by the more recent forms, delamination would persist as the mode of formation of the central mass or endoderm, even though a large blastocœl was present in the blastula.

I believe then that the phenomena occurring in *Metridium* are to be explained on the supposition that the Hexactiniæ are descended from forms whose ova possessed a relatively large amount of food-yolk. What we find in the Alcyonaria is the original condition, and the more typical delamination of the Hexactiniæ has been derived from this. In *Renilla* the central cells, heavily laden with food-yolk, are separated from the proportionately more protoplasmic ectoderm; later, a certain number of the central cells become transformed into the endoderm layer, while the rest degenerate and their contents serve as food for the developing embryo, being absorbed by its endoderm cells. So too, though to a less extent, in *Manicina*; the yolk is here much less in quantity, but still the same processes occur as in *Renilla*. In *Metridium* the yolk is much more reduced, so that the blastula cavity never becomes filled up, the delaminated cells becoming almost entirely converted into the definitive endoderm, a few only disintegrating, and giving rise to the granules of food-yolk which lie in the endodermal cavity.

2. THE FORMATION OF THE FIRST EIGHT MESENTERIES IN RHODACTIS.

The embryos of *Rhodactis Sancti-Thomæ*, when extruded by the parent, have, as stated above, from two to four perfect mesenteries. In shape they are pyriform, the mouth being situated at the narrower pole. The ectoderm is high, and shows a differentiation into the various kinds of cells usually to be found in that layer in the Actinians, but does not yet present that peculiar vacuolated appearance, due to an excessive development

of glandular elements, which characterizes the adult (McMurrich, '89 *a*). At the aboral pole there is a peculiar differentiation of the ectoderm which is not found elsewhere on the body. Immediately external to the mesogloea, which is well developed at this stage, there is to be seen in longitudinal sections of the embryo, a layer (Fig. 13, *n*) much more faintly stained with hæmatoxylin than either the mesogloea (*mg*) or the portion of ectoderm (*ec*) which lies superficial to it; it is crossed by numerous fine fibrils, in the meshes of which lies a clear, faintly granular substance, which does not stain at all, or but very faintly. The fibres are readily seen to be delicate prolongations of the mesogloea extending up into the ectoderm, and appearing in cross-section as deeply stained, round, homogeneous dots, and the matrix in which they lie is apparently composed of nerve-fibrils. It occupies the relative position held by the nerve-layer in adults, and it is probable that it is of that nature, although I have not been able to distinguish ganglion cells in it. In swimming, the aboral end is anterior, and the special development of sensory cells and a nerve-plexus at that pole is not surprising. The layer is distinguishable only for a short distance up the sides of the body, though probably it extends in a very much less developed condition all over the body. The special development at the aboral end must be considered a larval adaptation.

The endoderm consists of large, vacuolated cells, of the same nature as those found in the adult. They contain large numbers of Zoöxanthellæ, which occur, however, also in the ectoderm, and the large nematocysts, which I described as occurring in the endoderm of the adults, are present in the embryos only in the ectoderm. The endoderm cells in the younger stages completely fill up the central cavity, and show little or no arrangement into a definite layer. It seems probable from this that the formation of the germ-layers in this form differs somewhat from that described for *Metridium*, and more nearly approaches what occurs in the Alcyonaria and in *Manicina*.

In the youngest specimen obtained, the first pair of mesenteries is in process of formation. The number of embryos at my disposal is not sufficient to trace out fully all the processes concerned in the formation of these mesenteries, but I can merely deduce what is presumably taking place from conditions

which I find in a limited number of preparations; a more complete series is necessary to determine accurately the actual occurrences. We have, however, a description of a more ample series of embryos of *Manicina*, by H. V. Wilson ('88), and this furnishes a basis for understanding more disconnected stages. In a transverse section a little above the one figured (Pl. xiii, Fig. 14), the stomatodæum is seen to lie in close apposition to the column wall, there being no endoderm between its mesoglœa and that of the column. A little lower, as in the section figured, it has separated, and the mesentery is to be seen extending from it to the column. The axis of the stomatodæum is oblique to the long axis of the embryo, and therefore the section does not cut the stomatodæum transversely. The other mesentery of the first pair is seen also in this section, and it is noticeable that it has made its appearance independently of any apposition of the stomatodæum to its point of origin. In another specimen (Fig. 15), however, I find that such an apposition does occur in the manner described by H. V. Wilson for *Manicina* ('88), the stomatodæum passing over from the point of origin of the first formed mesentery, drawing this with it, to apply itself to the column wall at the region where the second mesentery is to be formed. It is certain, however, that this apposition of the stomatodæum is not necessary for the formation of the second mesentery, which may rise while the stomatodæum is still in its upper part in contact with the place of origin of the first mesentery.

There is as yet no development of mesenterial filaments. The stomatodæal ectoderm can be traced downwards in a series of transverse sections somewhat further on one side than on the other, but this is a necessary consequence of the obliquity of the stomatodæum to the long axis of the embryo. It is possible that there may be a slight elongation, but it is doubtful, since in the specimen from which Fig. 14 is taken, the stomatodæal ectoderm is cut in the sections lower down on the side on which the stomatodæum is in contact with the column wall than on the other, and if we consider this as indicating the formation of a filament, we will have the filament of the second mesentery more fully developed than that of the first, which is possible, but not to be expected.

The stomatodæum is now slung by two mesenteries. It lies

embedded in the endoderm, which, in the upper part of the body, does not yet show any signs of forming a definite layer, although below this, differentiation is accomplished, and a well-marked cavity exists. There are consequently in the upper part of the body as yet no intermesenterial cavities. In its upper part the stomatodæum is circular in section, but below it is elongated in the plane of the two mesenteries, which are situated nearly opposite each other.

The mesenteries of the second pair make their appearance simultaneously and without any migration and apposition of the stomatodæum to their line of origin. They are formed in the larger space, cut off by the two previously formed mesenteries, and gradually increase in size until they reach the stomatodæum, with which they unite.

The third and fourth pairs of mesenteries also appear simultaneously (Fig. 16), the third pair being in the interval between the mesenteries of the first pair; *i.e.* at the ventral surface of the embryo, and the fourth pair in the interval between the mesenteries of the second pair, or at the dorsal surface of the embryo. In the oldest embryo of *Rhodactis* which I possess these mesenteries are not yet perfect, and the longitudinal muscles have not developed, so that it is impossible to get definite evidence as to which pairs of mesenteries are really the directives. The manner of flattening of the stomatodæum in its lower part would suggest that each pair of directives consists of one mesentery of the first pair and one of the second; but it is to be noticed that the upper part of the stomatodæum does not take part in this flattening, which is probably to be considered as a temporary condition. It seems more probable that the future and permanent flattening of the stomatodæum is at right angles to the one existing in these early embryos of *Rhodactis*, and that the third and fourth pair of mesenteries will form the directives of the adult.

H. V. Wilson has described in *Manicina* a peculiar reflection of the ectoderm of the stomatodæum upon the external (*i.e.* normally endodermal) surface of that organ, and considers it to be concerned with the formation of the mesenterial filaments, with the exception of those of the first pair of mesenteries. In my specimens of *Rhodactis* the formation of the mesenterial filaments differs considerably from what has been described for

Manicina, the filaments of the first pair not appearing until a very much later stage of the development. In *Manicina* a down-growth of ectoderm pushing away the endoderm occurs while the stomatodæum is in contact with the column wall; in other words, the filament makes its appearance before the mesentery. In *Rhodactis* this is not the case, but the mesenteries are well developed, and the second pair well formed and even perfect, before the formation of the filaments begins. They appear to be down-growths of the stomatodæal ectoderm, extending down some distance below the lower end of the stomatodæum. It must be noticed, however, that the histological characteristics of the down-growths are not quite identical with those of the stomatodæum, and I cannot be certain that there is histological continuity.

In one specimen only was there any trace of the filaments of the second pair. Eight mesenteries were formed, two pairs only being perfect. Here I found not the slightest trace of any reflection of the ectoderm, but the filament, which was developed only in one of the pairs, was apparently simply a slight down-growth from the stomatodæal ectoderm. It developed in exactly the same manner as the filaments of the first pair, and there is not the slightest indication in this specimen of a reflection of the stomatodæal ectoderm. In a younger specimen, however, in which only the filaments of the first pair have begun to form, there is a peculiar formation of the lower end of the stomatodæum (Fig. 17), which is somewhat different from what Wilson describes. The stomatodæum on one side is split from below upwards for a short distance, and the ectoderm of the edge of the slit is reflected for a short distance. The slit figured is between the mesenteries of the second pair, and the ectoderm is reflected so as to enclose their extremities, but is not continued down at all to form mesenterial filaments. On the side of the stomatodæum, opposite the slit and between the mesenteries of the first pair, is a piece of reflected ectoderm, which is the upper edge of a slit similar to, but less in extent than, that of the other side. The reflected ectoderm of this side seems to be continuous with the mesenterial filaments of the first pair of mesenteries. In estimating the significance of these ectodermal reflections it must be remembered that even in adult individuals of various forms reflections of the lower

edge of the stomatodæum, similar to what I have just described, occur, and apparently have had something to do with the formation of the "Flimmerstreifen" of the filaments. These structures, however, have not made their appearance in any of the *Rhodactis* embryos I have had for study.

In another specimen, in which the second pair of mesenteries was just forming, I found some distance up upon the endodermal surface of the stomatodæum a patch of ectoderm (Fig. 18). It was not situated symmetrically, but was close to one of the mesenteries of the first pair. I thought at first that this might be a piece of reflected ectoderm which had migrated upwards some distance. It had evidently, however, completely lost its continuity with the stomatodæal ectoderm below, while on the other hand the mesogloea, which ought to have occurred below it, separating it from the stomatodæal ectoderm, was not present, so that it was in direct continuity with the stomatodæal ectoderm, and it seems as if there had been an escape of ectoderm upon the endodermal surface of the stomatodæum through a perforation of the mesogloea. I am strongly inclined to take this to be a malformation, especially as we have already seen that the filaments of the second pair of mesenteries develop only after the mesenteries have become perfect, there being therefore no necessity for an ectodermal reflection, even though they are developed from that layer.

The evidence, so far as it goes, indicates that in *Rhodactis* there is no reflection of the stomatodæal ectoderm in connection with the formation of the mesenterial filaments, and in *Aulactinia* the evidence points to the same direction. The question arises as to whether the arrangement in *Manicina* is the more primitive, or whether the formation of the filaments is typically postponed until the mesenteries have become perfect. The latter seems to me to be the more primitive method, since in all probability the mesenteries are phylogenetically older structures than the filaments. In embryos of *Edwardsia*, too, I find that the filaments, with the exception perhaps of those of the first pair of mesenteries, do not appear till the eight mesenteries have become perfect. This has an important bearing upon the question, since *Edwardsia* must be regarded as representing an ancestral condition of the Hexactinians. I think, then, that the peculiarities of *Manicina* are secondary,

and are perhaps to be referred to the early appearance of the mesenterial filaments.

It must be remembered, however, that there are different opinions as to the nature of the mesenterial filaments in the Actinozoa, some authors considering them to be endodermal and others ectodermal. As stated above, they *appear* to be ectodermal in *Rhodactis*, at least the median portion, which is all that is developed, does. At the same time I must refrain from expressing any certainty on this point, (1) on account of the slight difference in histology between the ectoderm of the stomatodæum and of the filaments, and (2) because it seemed, in the case of a very young filament of the second pair of mesenteries, that, in a series of transverse sections, there was a very short streak of indifferent cells intervening between the stomatodæum and the filament, interrupting the continuity of these structures. If this be the case, it would seem that the filaments, or at least their median portion, were endodermal, which seems to be the case with those of *Aulactinia*, to be described later.

3. LATER STAGES IN AULACTINIA.

In the youngest embryo of *Aulactinia* which I possess, the endoderm presents certain peculiarities which allow us to infer the mode of development in the earlier stages to a certain extent. There are already eight perfect mesenteries, only two of which — those of the first pair — are provided with mesenterial filaments. The endoderm has arranged itself into a somewhat definite layer, but lying scattered about in the body cavity of the embryo are numerous somewhat large cellular elements and yolk-granules. The endoderm is in a stage of differentiation which corresponds fairly well with that represented in *Renilla* by E. B. Wilson ('83) in his Fig. 125, Pl. 57. From the occurrence of these cellular elements, it seems probable that the formation of the germ-layers took place in a manner similar to what is found in the Alcyonarians, rather than like what I have described for *Metridium*.

The stomatodæum in these youngest larvæ is elongated throughout its entire length in a direction at right angles to the elongation noticed in its lower part in *Rhodactis*, so that a line drawn in the axis of the elongation passes through the inter-

mesenterial spaces between the third and fourth pairs of mesenteries. Upon the mesenteries the longitudinal muscle processes have begun to appear. The manner of their arrangement may be better seen, however, from an older specimen, a figure of which is given (Pl. XIII, Fig. 19). It will here be seen that the embryo is bilaterally symmetrical, the line separating the antimeres passing between the mesenteries of the third and fourth pairs, and therefore in the direction of the elongation of the stomatodæum. The mesenteries of the third and fourth pairs are seen to have their longitudinal muscles on the faces which are turned away from each other, and accordingly correspond to the directives of the adult. The mesenteries of the first and second pairs do not form a pair in the same manner as the directives, but each mesentery of these pairs has its longitudinal muscles upon the face which is directed towards the third pair of mesenteries, which we may term the ventral directives.

We have then in this stage an arrangement which exactly reproduces the condition permanent in the *Edwardsiæ*, and which may therefore be termed the *Edwardsia* stage. The significance of this from a phylogenetic standpoint I have already elsewhere pointed out ('89), and do not intend to enter into this question here, hoping to return to it with a discussion of other investigations on the subject in a future number of these "Contributions."

The next stage witnesses the formation of two additional pairs of mesenteries, which make their appearance simultaneously upon either side of the mesenteries of the first pair (Fig. 19). These grow rapidly, finally reaching the stomatodæum, and developing their longitudinal muscles. Twelve perfect mesenteries are then present. The mesenteries of the fifth pair have their longitudinal muscles on the face which is turned towards the mesenteries of the first pair, and those of the sixth pair have them on the face which is turned towards the mesenteries of the second pair, *i.e.* upon the dorsal surface in each case. When these youngest mesenteries have become perfect we have a stage which exactly resembles the condition permanent in *Halcampa*, and which, therefore, may be termed the *Halcampa* stage.

The mesenteries are arranged in six pairs. Two of these, occupying the dorsal and ventral surfaces, are formed each of

mesenteries which have arisen simultaneously, or nearly so, in corresponding regions of each antimere of the bilaterally symmetrical embryo, and have their longitudinal muscles upon the faces turned away from each other. The other four pairs have, however, a very different constitution. Each consists of one mesentery formed previously to the directives, and one formed after their completion. Whereas in the case of the directives the mesenteries forming each pair are homochromous in their formation; in the lateral pairs, the mesenteries of each pair are heterochromous. These heterochromous pairs differ from the directives in the position of the longitudinal muscles which are on the adjacent faces of each pair.

In succeeding stages as Lacaze-Duthiers pointed out ('72) the appearance of new mesenteries is governed by a law different from that which operated up to the conclusion of the *Halcampta* stage. Up to this period the mesenteries made their appearance bilaterally, one on each side of the dorso-ventral line, the tendency towards bilateral symmetry being the governing force which determined the manner of their development. In the stages which now supervene, the new pairs of mesenteries make their appearance as if governed by a radial symmetry, developing simultaneously in the intervals between each of the six *Halcampta* pairs, the mesenteries of each pair forming contiguously. In this way a stage with twelve pairs of mesenteries is formed, having all the characteristics of a Hexactinian. Embryos showing the formation of this second cycle of mesenteries are the oldest that I possess.

As regards the formation of the mesenterial filamentals my observations on *Aulactinia* are somewhat more definite than those on *Rhodactis*, though at the same time I do not regard them as perfectly conclusive. Taken, however, into consideration with what I have seen in adult Actinians, and with the very definite observations of E. B. Wilson on the development of the filaments in the Alcyonaria ('83 and '84), I am inclined to believe that the median streak of the mesenterial filaments of *Aulactinia* are developed from the endoderm.

As already stated, in the youngest *Aulactinia* embryo mesenterial filaments are present only on the mesenteries of the first pair, so that I have ample material illustrating their formation on the other mesenteries. In all cases it is the median streak

(*Nesseldrüsenstreif*) which is first formed, and for some time it is the only portion of the filament which is present. I shall consider its formation first, reserving a description of the formation of the lateral streaks (*Flimmerstreifen*) until later.

In an embryo in which the second cycle of mesenterial pairs is making its appearance, the first four pairs of mesenteries are already provided with fully formed filaments. The fifth and sixth pairs are just forming them, while in the mesenteries of the second cycle, which are yet quite small, there is no trace of them. It is certain that the reflection of stomatodæal ectoderm described by H. V. Wilson does not exist. The mesenteries of the fifth and sixth pairs have reached the stomatodæum and have fused with it for about the upper half of its length; below, however, they are still separate from it. In a section which passes through the stomatodæum immediately below the point of separation of the mesentery from it (Fig. 21), it can be seen that no ectoderm exists upon its outer surface, while in sections lower down (Fig. 22), the mesenterial filament is seen. There is therefore no connection between the stomatodæal ectoderm and the cells forming the free edge of the mesentery. The conclusion is evident that the median streak of the mesenterial filament is a product of the endoderm.

I have verified this result in the case of the third mesenteries in younger larvæ, and have no reason to doubt its accuracy. The statements of H. V. Wilson ('88) and E. B. Wilson ('84) are both so definite, and at the same time so absolutely opposed to one another that it is of importance to determine, by the study of a number of forms, what actually takes place. It does not seem probable that structures having the same morphological and physiological characteristics should be derived in such nearly related forms as the Alcyonaria and Hexactinians from two different germ-layers, and the question seems to be as to the correctness of the interpretation of the observations made by the authors mentioned. What I have found inclines me to accept the observations of E. B. Wilson.

The filaments, when first formed, consist, as stated above, solely of the median streak. This is small at first, and differs considerably from the structure of older filaments, as can be seen from a comparison of Figs. 20 and 23. In the younger filament (Fig. 20) the cells are elongated, differing markedly

from the endoderm cells in the vicinity, but do not show any very decided differentiation into gland and nettle cells. The older filament (Fig. 23), on the other hand, has numerous nettle cells which stand out prominently in specimens stained with hæmatoxylin, and gland cells are quite numerous. Upon each side of the filament the endoderm cells are heightened so as to form a very decided wing upon either side, and there is no trace of any extension of the filament cells out upon this wing. The appearance which is seen in Fig. 21 is due to the section having passed through a bend of the filament. Owing to the contraction of the embryos the filaments are much contorted, and sections frequently give an appearance as if several filaments branched out from the margin of the mesentery. A reconstruction in wax of a mesentery and its filament showed, however, that this appearance was deceptive, and was due to the bending and twisting of the filament.

There is one bend, however, which is constant in all the embryos, and occurs a short distance below the point where the mesentery leaves the stomatodæum. The edge of the mesentery is here bent upon itself, so that transverse sections show a portion of the mesentery attached to the stomatodæum, its edge being turned towards the edge of the rest of the mesentery (Fig. 24). This bend is important since it is upon the portion that lies morphologically above it, that the lateral streaks of the filaments are developed.

In the embryo from which Figs. 21 and 22 were taken, the lateral streaks had developed upon the first and second pairs of mesenteries. In these in a section taken just below the point where the bend mentioned above occurs (Fig. 24), the median streak (*ms*) of the filament is well differentiated, and the two endodermic wings may be seen on that portion of the filament which is below (morphologically speaking) the bend. On the upper part of the mesentery, that which in the section is attached to the stomatodæum, quite a different structure is found. The mesogloea of the mesentery here branches into three processes at its free edge, and these support the three lobes of the mesenterial filament. The two lateral lobes (Flimmerstreifen) (*ls*) are readily to be made out from the dark stain which they take, and on tracing them onwards towards the lower edge of the stomatodæum the epithelium which forms them can be seen

to pass, without any solution of continuity, directly into the stomatodæal ectoderm. It would seem, then, that the lateral streaks of the filaments are *ectodermal* in their origin, and consist of two down-growths of the stomatodæum along the sides of the mesenteries close to their free edge.

The third or median process of this portion of the filament has a quite different structure. It does not at all resemble the lateral streaks of the filament, neither is it similar to the median streak (Nesseldrüsenstreif), which is found lower down. It appears to be more or less undifferentiated, and resembles the endoderm in its structure rather than the ectoderm, although the similarity is rather obscured by the presence in the general endoderm of large numbers of *Zoöxanthellæ*, which are absent in the median process. I believe it to be endodermal in its nature, and to be the persisting endoderm which covered the free edge of the filament before the lateral streaks made their appearance, the endoderm at the sides of the free edge only being pushed down or replaced by the ectodermal Flimmerstreifen.

I have not considered it necessary to review the literature upon the formation of the mesenterial filaments; this has been sufficiently done in the papers of E. B. Wilson and H. V. Wilson, already referred to. It may be seen that my results as stated above agree perfectly with those obtained by E. B. Wilson ('84), and fully bear out the suggestion advanced by him that the Flimmerstreifen of the Hexactinian filament is equivalent to the dorsal mesenteries of the Alcyonaria, and that the median streak (Nesseldrüsenstreif) is equivalent to the six ventral filaments of members of that group. On the other hand, I am utterly at variance with H. V. Wilson on the question of the origin of both portions of the filament. Wilson homologizes the simple unlobed filament of *Manicina* with the entire trilobed filament of such a form as *Aulactinia*. He supposes that in primitive forms the filament was simple and unlobed, and that by a process of physiological differentiation became divided into a central glandular and lateral respiratory portions. This theory I cannot accept. The mere fact that I believe in the origin of the two portions of the filament from different germ-layers precludes its acceptance; but in addition the manner of formation of the lateral streaks shows them to

have had an independent origin from the median streak. If we may judge from the individual development, the median streak was the first to make its appearance in the ancestors of the Actinozoa, and they remained with such simple glandular filaments, formed by a differentiation of the endodermal cells along the free edges of the mesenteries, for some time. Later, the ciliated respiratory portions of the mesentery developed by a down-growth of the stomatodæal ectoderm, and were at first confined to the upper portion of the mesentery where the glandular streak was not developed, but later, extending their limits, they came to overlap the glandular portion, and thus the filament became trilobed throughout a portion of its length.

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October 14, 1890.

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EXPLANATION OF PLATE XIII.

FIGS. 1-12 are of *Metridium marginatum*.

FIG. 1. Immature ovum taken from the ovary.

FIG. 2. Commencement of the first cleavage.

FIG. 3. First cleavage about half completed.

FIG. 4. Flattening out of spherules at conclusion of the first cleavage.

FIG. 5. Four-celled stage.

FIG. 6. Eight-celled stage.

FIG. 7. Early blastula; figure drawn from preserved ovum.

FIG. 8. Optical section of free-swimming blastula.

FIG. 9. Optical section of blastula, just before the breaking through of the mouth, showing the depression at the posterior pole. Drawing made from preserved specimen.

FIG. 10. Optical section of gastrula stage; drawn from a preserved and cleared specimen.

FIG. 11. Section through blastula, 48 hours old. Camera. Zeiss, D, 2.

FIG. 12. Section through embryo, showing the formation of endoderm by delamination. Camera. Zeiss, D, 2.

FIGS. 13-18 are from *Rhodactis Sancti-Thomæ*.

FIG. 13. Portion of longitudinal section through anterior (aboral) pole of young larva. *en*=endoderm, *mg*=mesogloea, *n*=nerve-layer, *ec*=ectoderm. Camera. Zeiss, J, 2.

FIG. 14. Transverse section through larva, showing formation of second mesentery. Camera. Zeiss, A, 4.

FIG. 15. Transverse section through larva, showing formation of second mesentery by apposition of stomatodæum to its line of formation. Camera. Zeiss, A, 4. The details of the endoderm in this and the three succeeding figures are not completed.

FIG. 16. Transverse section, showing the formation of the third and fourth pairs of mesenteries. Camera. Zeiss, A, 4.

FIG. 17. Transverse section through larva, showing reflection of stomatodæal ectoderm. Camera. Zeiss, A, 4.

FIG. 18. Transverse section through larva, showing ectoderm on outer surface of stomatodæum, apparently having broken through the mesogloea. Camera. Zeiss, A, 4.

FIGS. 19-24 are from embryos of *Aulactinia stelloides*.

FIG. 19. Transverse section of larva, showing the formation of the fifth and sixth pairs of mesenteries. Camera. Zeiss, A, 2.

FIG. 20. Transverse section of mesenterial filament of the third pair of mesenteries of larva with only four pairs of perfect mesenteries. Camera. Zeiss, D, 2.

FIG. 21. Transverse section through a mesentery of the fifth pair, just below the point where it separates from the stomatodæum. *st*=stomatodæal ectoderm, *en'*=endoderm of mesentery, *ec*=ectoderm of column wall. Camera. Zeiss, D, 2.

FIG. 22. Transverse section through same mesentery and the adjacent one of the first pair taken 0.08 mm. lower down. Lettering as in preceding figure, except *ms*=median streak of mesenterial filament. Camera. Zeiss, D, 2.

FIG. 23. Transverse section of mesenterial filament of the first pair, from larva from which Fig. 20 was taken. Camera. Zeiss, D, 2.

FIG. 24. Transverse section of mesenterial filament of mesentery of the first pair, taken just below the bend of the mesentery, from larva with twelve perfect mesenteries. Lettering as in Fig. 22, except *ls*=lateral streaks of mesenterial filament.



ON INTERCALATION OF VERTEBRÆ.¹

G. BAUR.

WHEN we have two nearly related animals, which have a different number of segments, the question arises, What is the origin of this difference? There are two possibilities in regard to the two forms; one may be derived from the other, either by increasing or decreasing the number of segments. In each of these two cases we have different possibilities. In the case of increase, the new segments may either be developed by intercalation, or by division of the original segments, or by addition at the caudal end of the animal. In the case of decrease, we can think of excalation, union, or loss of segments at the caudal end.

Most morphologists are inclined to the opinion of addition or subtraction of segments at the distal end. But there are others, like Jhering and Albrecht, for instance, who adopt intercalation.

Let us now consider some cases. In most of the higher vertebrates we have a sacrum which is united to the vertebral column by sacral ribs: this sacrum establishes a more or less fixed point in the vertebral axis. We distinguish presacral and postsacral, or caudal vertebræ. The increased number of presacral vertebræ may be produced, either by intercalation of new vertebræ, or by movement of the sacrum backwards. The decreased number may be the result of excalation, or of the movement of the sacrum forwards.

Cases of the movement of the sacrum have very often been described, and quite a number have come under my own observation. Positive cases of intercalation, however, have seldom been recorded.

Fürbringer² discusses the question in the chapter, "Über die Verschiebung (Wanderung) der Extremitäten," of his great

¹ Paper read before the American Morphological Society, Boston, Dec. 29, 1890.

² Fürbringer, Max: *Untersuchungen zur Morphologie und Systematik der Voegel*, Amsterdam, 1888, Vol. II, pp. 972-991.

work on the morphology of birds. He speaks also about the case of intercalation published by Albrecht (in the Bull. Mus. Roy. d'Hist. Nat. de Belgique, II, 1883). Page 975 he says:—

“Für die Verschiebung der Gliedmassen und die dadurch beeinflusste Umbildung der Wirbel sind manche Argumente bisher beigebracht worden, für die Inter- und Excalation habe ich sie dagegen bis jetzt vermisst.

“*Albrecht* statuirt Segmentvermehrungen durch Theilungen der Ursegmente, entscheidet sich somit für die Annahme einer Interpolation in embryonaler Zeit. Nach meinen bisherigen Anschauungen hatte ich gegen die Möglichkeit einer Interpolation nichts einzuwenden, aber bei dem völligen Mangel irgend welches sie stützenden Argumentes konnte ich ihr nur eine rein theoretische oder begriffliche Bedeutung, aber keine Wahrscheinlichkeit zuerkennen.”

Albrecht examined a skeleton of *Python sebae* in the Museum at Brussels. Between the 194th and 197th vertebræ, he found a complex of anchylosed vertebræ: this contained on the right side two vertebral elements, one foramen intervertebrale, and two ribs; on the left side three vertebral elements, two foramina intervertebralia, and three ribs. Albrecht considers the supernumerary vertebra on the left side as intercalated. Fürbringer, on the other hand, is inclined to regard it as the result of some pathological process, which reduced the right half of the vertebra. He says: “Welcher Art und Veranlassung dieser pathologische Process gewesen und wann er stattgefunden, kann ich natürlich nicht entscheiden, halte auch diese Frage für eine nebensächliche; aber die namentlich auf der Dorsalansicht sehr auffallende unregelmässige Schraegstellung des fraglichen Halbwirbels, der Umstand, dass er kein reiner Halbwirbel ist, sondern im Bereiche des Körpers und im Bereiche der Neurapophysen noch Rudimente der rechten Seite darbietet, endlich die anchylosirung der 2¹ Wirbel, während die Wirbelsäule sonst sehr ausgebildete Gelenke besitzt machen es mir unmöglich hier an einen normalen Fall von Wirbelinterpolation zu denken.”

I think I shall be able to show that Albrecht was correct in his interpretation. Already Koken, in writing a review of Albrecht's paper (in one of the numbers of the “Neues Jahrbuch für Mineralogie,” which I have not at hand), states that he has

observed similar cases as Albrecht's in *Tropidonotus*. R. Owen¹ has also described a case of the same nature in the skeleton of a *Python tigris*, of which he says: "Anchylosis has occurred between the 148th and 149th vertebræ. The 166th and the 167th vertebræ have been more completely and abnormally blended together, so as to seem but one vertebra on the left side, where that half of the neural arch and spine have completely coalesced, whilst on the right side each vertebra supports its own rib. A similar abnormality occurs between the 184th and 185th vertebræ."

If intercalation takes place at all, we ought to expect traces of it in such forms as show a great increase in the number of vertebræ, for instance, snakes, different groups of lizards, and Plesiosaurs. In looking over such material I have found some additional cases.

In a specimen of *Pelamis bicolor* (No. 763, Yale University Museum) I find the 212th vertebra simple on the left side, double on the right side; it bears one rib on the left, two ribs on the right side. Exactly the same condition I have observed in a cervical of a Plesiosaur, *Cimoliasaurus plicatus*, No. 48,001 of the British Museum, London. One side has one, the other two ribs. Mr. R. Lydekker² makes the following remark about this vertebra:—

"The centrum of a small and malformed cervical vertebra, from the Oxford Clay near Oxford. This specimen is immature, and on one side is divided into two portions, each with its distinct costal facet."

I do not doubt at all that in all these cases we have examples of incomplete intercalation; and I am convinced that this intercalation is the result of the division of myotomes, as expressed by Albrecht. The possibility of such intercalation I have expressed already in No. 306 of the "Zoologischer Anzeiger," 1889, where I said: "Ich glaube, dass eine Verschmelzung oder Spaltung von Myomeren auch schon während der Anlage des Embryo möglich ist. Mein Freund A. Böhm in München theilte mir mit, dass er verschiedene Anzeichen von Spaltung von Myomeren beobachtet habe."

¹ Descriptive Catalogue of the Osteological Series contained in the Museum of the Royal College of Surgeons of England, Vol. I, p. 123, London, 1853.

² Lydekker, Richard: Catalogue of the Fossil Reptilia and Amphibia in the British Museum, Part II, London, 1889, p. 238.

Of course the direct proof of splitting of myotomes could only be given by the study of the living embryo, which, if possible at all, is exceedingly difficult. If we have the complete segments in the embryo or in the adult animal, we cannot decide whether they consisted originally of a single myotome, or whether they are the result of division. For instance, in the peculiar consolidation of vertebræ in snakes, mentioned by Owen, and also observed by me at different times, we do not know whether this consolidation is the result of real union of two segments, or of partial division of one segment. At least we may adopt one explanation just as well as the other. I am inclined, however, to accept partial division in these cases. A great number of observations is necessary to see in what relations the frequency of such consolidation stands to the increased number of segments; in other words, whether such complexes are more frequent in animals with the number of segments considerably increased, as it appears to-day, than in such which have a relatively small number.

By very careful study and comparison of the structure of the single vertebræ, however, it is sometimes possible to determine whether we have a case of intercalation or not. It is well known that the typical number of the presacral vertebræ in the living Crocodilia is twenty-four; there are two sacrals: the first caudal is peculiar, by being biconvex. In a specimen of *Gavialis gangeticus* I found twenty-five presacral vertebræ.¹ As in all living Crocodiles the first caudal vertebra is biconvex; but in this case it is the twenty-eighth, in the other the twenty-seventh. Is it not evident, therefore, that at some place between the occipital condyle and the first caudal a new vertebra has been inserted? By careful comparison I find that this new vertebra has been intercalated between the ninth and tenth.

A similar case I have observed in *Heloderma*. In this lizard the first caudal vertebra has also a peculiar character. The small rib connected with it is perforated; this perforation is absent in the other vertebræ. By this peculiarity the first caudal vertebra is distinguished from the rest. Four specimens of *Heloderma* show the following condition. In the first speci-

¹ G. Baur: *Anzahl der praesacralen Wirbel der Crocodilia*. Zool. Anz. No. 238, 1886.

men the first caudal is the thirty-sixth (*Heloderma horridum*);¹ in the second, the thirty-seventh (*Heloderma horridum*, observed by me); in the third, the thirty-eighth² (*Heloderma suspectum*); in the fourth it is the thirty-ninth (*Heloderma suspectum*, Clark University). We have, therefore, four variations in four specimens. There seems to me very little doubt that this difference in the number of vertebræ is produced by true intercalation.³

By these few but characteristic examples I believe to have given positive evidence that intercalation of segments takes place in vertebrates. I do not doubt that further examination of more material will bring out more cases. What is necessary to do, is to examine a great number of specimens of the same and allied species of such forms as show an unusual increase of segments, like the Varanidæ, Scincidæ, Anguidæ, Amphisbænidæ, Snakes, and so on. The embryology of such forms would probably give important evidence, because we may expect to find indication of myomeric division.

My opinion is that in the increase of the number of segments not only in vertebrates, but also in invertebrates, intercalation has played a much greater rôle than is generally admitted. At the same time I admit addition of segments at the distal end, as well as occasional *slight* migration of the shoulder girdle and pelvis in both directions. The question is an important one, and I hope that some embryologist may take up the subject for further study.

This question of increase of the number of segments is a very interesting one from the standpoint of the evolutionist. It is evident that intercalation can only take place in the very early life of the embryo, when the myotomes are forming, and that it is absolutely impossible that new segments can be intercalated through any effort and exercise of the animal

¹ Troschel, F. H.: *Über Heloderma horridum*. Wieg. Arch. f. Naturg., Jahrgang 19, Vol. I, Berlin, 1853, pp. 294-315. I am indebted to Mr. S. Garman, Cambridge, for looking up this reference for me, the Journal not being at hand.

² Shufeldt, R. W.: *Contributions to the Study of Heloderma suspectum*. Proc. Zool. Soc., London, 1890, p. 214 (the peculiar character of the first caudal is not mentioned by Dr. Shufeldt).

³ Whether this intercalation is produced by division of myotomes, or by addition of myotomes from the beginning, I do not know; both ways are possible. I am convinced that in a great number of cases intercalation takes place by adding new segments in the embryo without division.

during the later period. *The disposition to increase the number must be, therefore, in the germ itself.* The question is then, Is the increase of the number of segments "accidental," and are forms which show this "accidental" increase of segments preserved through natural selection; or is this tendency to increase the number of segments common to all individuals, not appearing accidentally, but rather as the result of a definite stimulus? I can only adopt the latter possibility.

But how is it in this case? Many animals increase the length, that of the neck for instance, not by addition of new segments, but simply by elongating the single segments present. For instance, the Giraffe among mammals, Chelys, Chelodina, Diroschelys, Hydromedusa among the tortoises. How is it that the Plesiosaur elongates its neck by adding new segments, and the Tortoise by stretching the single segments? Here we are before a difficult question. It is clear the long neck of the Giraffe and of some of the Tortoises develops during the evolution of the animal, but this tendency must be potentially in the germ. It is again the tendency to elongate the neck which is impressed on the germ, but in a different way. An interesting case of addition of segments is offered by the Sirenians. The Sirenians are the only mammals, the Cetaceans excepted, which show an increase of phalanges (four instead of three); a fourth phalange is added in some digits at the distal end, but this takes place during the postembryonic life of the animal, the embryo having only three phalanges.

I am not able at present to give any explanation for these phenomena, but I thought it worth while to mention them in this connection.

NEUROBLASTS IN THE ARTHROPOD EMBRYO.

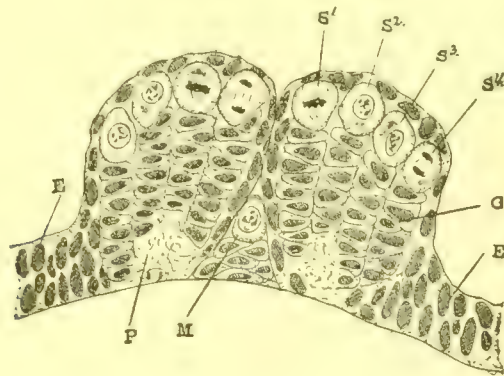
WILLIAM M. WHEELER.

As some months will probably elapse before I shall be able to complete my study of *Xiphidium ensiferum*, I take advantage of the opportunity kindly offered me by Professor Whitman to publish a few observations bearing on the development of the nervous system of Arthropods.

In *Xiphidium* the nervous system begins to make its appearance before the elongate blastopore closes and the fold of the amnion and serosa envelops the head—at a time, therefore, when the embryo is still in what I have called the “slipper” stage. There may then be seen, scattered over the surface of both procephalic lobes a number of pale circular spots, each surrounded by a ring of the small cells forming the ventral plate. The embryo still consists of a single layer of elements except beneath the blastopore, where the meso-entoderm is differentiating. In sections the pale circular spots are seen to be due to centres of proliferation, each consisting of a few enlarged cells. Surface study is rendered difficult as soon as the envelopes have enclosed the head, and it is not till the embryo has grown and stretched the overlying amnion that the surface again becomes clearly visible. In the meantime the nervous system must be studied in sections.

Beginning with the ventral nerve-cord, I find that the ganglia arise as paired thickenings of the ectoderm in the manner so often described for Arthropods. The thickenings are the lateral cords (“Seitenstränge”)—the region between them, marked on the surface by a groove, is the median cord (“Mittelstrang” of Hatschek). Carefully made transverse sections through either lateral cord are seen to consist in early stages of two kinds of ectoderm elements: smaller cells with rather deeply stainable elongate oval nuclei and four large succulent cells with pale spherical nuclei. These four large cells, the *neuroblasts*, lie side by side just beneath the smaller ecto-

derm elements in a plane parallel to the surface of the yolk and the outer surface of the body. As every transverse section through the ventral nerve-chain in this stage contains approximately four of these huge cells, I conclude that the embryo presents eight longitudinal rows of neuroblasts. The rows extend from the mouth to the anus, and are most clearly differentiated anteriorly. When sections of the head are examined it is found that the sporadic clusters of cells forming the pale surface spots of the younger embryo have arranged themselves as neuroblasts in eight rows in either procephalic lobe. Four of these rows—those on either side of the stomodæal orifice and directly continuous with the rows of the ventral cord—give rise to the brain proper, while four shorter lateral rows form the optic ganglion. The retinal ganglion is delaminated very early from the outer edge of the procephalic ectoderm. From the first its elements do not resemble the neuroblasts and appear to multiply irregularly.



I give a slightly diagrammatic figure of a cross-section through the posterior portion of a basal abdominal ganglion. It is taken from an embryo somewhat older than the one just described. The two lateral cords have been made to bulge out beyond the general surface of the ectoderm by the proliferation of the neuroblasts (S^1 – S^4), each of which now surmounts a pillar of smaller elements. These, the future ganglion cells, are budded off from the inner ends of the neuroblasts and become cuneiform from mutual pressure. There is a marked contrast between the terminal and daughter cells, not only in size and shape, but also in intensity of stain.

The cytoplasm of the neuroblasts is very pale and finely granular, and their nuclei are pale and refractive, while the daughter cells stain much more deeply throughout and thus resemble the elements of the integumentary ectoderm (*E*). The polar axes of the neuroblast spindles seem always to be directed at right angles to the surface of the body in embryos of this stage. The "Punksubstanz" (*P*) makes its appearance in the bases of the lateral cords, which in the section figured are separated by a pyramidal mass of cells, the median cord. Heading this mass of cells is another neuroblast (*M*) apparently pushed below the surface by the bulging of the lateral cords. Owing to the shape of the space to which the terminal cell is confined, its daughter cells cannot arrange themselves in a straight column, but lie heaped up somewhat irregularly. While the four lateral neuroblasts represent the cross-section of four continuous longitudinal rows of cells, the median cord neuroblasts are isolated elements which arise intersegmentally, but soon move forward between the two connectives, and finally come to lie just back of the posterior commissure in each segment. At a still later stage each is incorporated together with the mass of cells to which it has given rise in the posterior part of a ganglion. Inasmuch as each postoral ganglion appears to be provided with one of the median cord cells, these elements constitute a ninth unpaired and interrupted row of neuroblasts extending like the lateral rows from the mouth to the anus. The interruptions occur at the points where the commissures are formed and at the intercommissural spaces. I have not yet been able to find median cord neuroblasts in very young embryos, but I do not doubt that they differentiate from the primitive ectoderm at the same time as the neuroblasts of the lateral cords.

The brain and optic ganglia arise as strings of cells budded off from the sixteen rows in the same manner as the ventral ganglia originate from the eight rows of neuroblasts.

In a stage succeeding the one figured, the daughter cells (*G*) of the neuroblasts themselves divide, the axes of their spindles lying at right angles to the axis of the mother spindle. Thus each neuroblast becomes the end of a pillar consisting of from two to four rows of ganglion cells which have arisen by division of the elements in the original single row.

Like all the cells which they produce, the neuroblasts are finally enclosed by the outer neurilemma. By the time the embryo has undergone revolution and has enveloped nearly the whole of the yolk, the ganglia are so crowded with cells that the neuroblasts have very little space in which to proliferate. They continue, however, to bud off new cells, notwithstanding the short rows thus formed are forced to lie parallel instead of at right angles to the surface of the ganglion. During this process the neuroblasts gradually grow smaller, till they finally become indistinguishable from their progeny, the ganglion cells.

I would emphasize the definite number and arrangement of the neuroblasts in *Xiphidium*, because I believe the eight rows of the lateral cords to be the homologues of the two rows of cells derived from the neuro-teloblasts in Annelids. The fact that there are only two rows in Annelids, whereas there are eight in *Xiphidium*, can constitute no very serious obstacle to this homology, since we have only to suppose that in the annelid-like forms from which the Tracheates are descended, the pair of primitive neuro-teloblasts divided twice to form four pairs of proliferating centres for the longitudinal rows of neuroblasts. It is not improbable that forms with more than a single pair of neuro-teloblasts may yet be found among existing Annelids. The homology here advocated is rendered more probable by the fact that the embryo *Xiphidium* does not conform to Graber's "law" of metameric segmentation, but, like Myriopods and Annelids, grows by the intercalation of segments in front of the anal plate. The neuroblasts in the proliferating zone at the end of the body are probably true teloblasts, since they bud off the neuroblasts of the different segments. They may be called primary neuro-teloblasts to distinguish them from the secondary neuro-teloblasts that produce the strings of ganglion cells.

It is more difficult to account for the interrupted series of median cord neuroblasts. Do they represent the remnants of a single unpaired row or of a pair of rows? Are they secondarily derived from one or both of the inmost rows of lateral cord neuroblasts (S^1), or have they had an independent origin? These are questions which I cannot yet answer. The interruptions in the median series are probably due to the formation of the commissures; primitive conditions prevailing only in

the intersegmental region where the original ectodermic layer has been less affected by neural differentiation. The absence of median neuroblasts in front of the mouth is easily accounted for by the high degree of concentration and the lack of intersegmental spaces in the three pairs of ganglia that constitute the brain.

The development of the ganglia from neuroblasts is by no means peculiar to *Xiphidium* among insects; it is quite as distinct in other Orthoptera. In the common locust (*Melanoplus femur-rubrum*) I can detect the same number of rows of proliferating cells both in the ventral nerve-cord and in the optic ganglia and brain. In *Xiphidium* and *Melanoplus* all stages in the proliferation of the neuroblasts may be observed in a single embryo; in the brain the columns of cells attain a considerable length before the neuroblasts in the terminal segments have budded off more than one or two cells.

Blatta germanica presents essentially the same conditions as *Melanoplus*.

The "ganglioblasts" of *Doryphora decemlineata* described in a former paper are the same as the cells which I now call "neuroblasts." Re-examination of my preparations has convinced me that in this insect also the number of rows is approximately four for either lateral cord. I fail, however, to detect a definite string of cells extending inward from each terminal cell; still there can be no doubt that the irregularly arranged ganglion cells are budded off from the neuroblasts. *Doryphora*, a highly modified form even among Coleoptera, would be expected to present in the development of its nervous system characters less primitive than those of the Orthoptera.

Neuroblasts in insect embryos have been described and figured by other investigators, but no one, to my knowledge, has called attention to their definite number and to their striking similarity to Annelid neuroblasts. Korotneff, in his paper on *Gryllotalpa*,¹ figures four neuroblasts in the lateral cord in two cases (Pl. XXX, Fig. 60; Pl. XXXI, Fig. 61). He also describes the manner in which they differentiate from the primitive ectoderm and proliferate to form ganglion cells (p. 589). His figures are taken from embryos too old to show

¹ Zeitschr. f. wiss. Zool., Bd. 41, 1885.

the single band of cells proceeding from each neuroblast; the daughter cells having divided, so that each teloblast surmounts three or four rows of small cells. Graber, in a recent paper,¹ calls attention to the huge cells that give rise to the ganglionic thickenings in the Dipter *Lucilia* and in the Coleoptera *Lina* and *Melolontha*.

Of great interest in this connection are the figures given by Dr. Patten in his last paper.² The nervous system of the young scorpion embryo is represented as covered with peculiar "sense-organs," which, strange to say, are arranged in four irregular rows in either lateral cord of the ventral nerve-chain. A single large "sense-organ" occurs in the middle of the median cord portion of each segment, and hence nearly corresponds in position to the median neuroblast of *Xiphidium* after it has moved forward between the connectives. The "sense"-spots of the scorpion are very similar to the spots which in the procephalic lobes of the Locustid mark the small areas where neuroblasts are differentiating from the primitive ectoderm. Patten gives no description of these "sense-organs" in his text, and the cut representing a transverse section through a ventral ganglion is on too small a scale to show the surface cells distinctly. Laurie³ figures a transverse section through the procephalic lobes of an embryo in a stage that I take to correspond to one of the young embryos figured by Patten. In this figure (Pl. XV, Fig. 25) the ectoderm shows in the arrangement of its nuclei a peculiar unevenness which may account for the circular spots figured by Patten, but which would seem to have no connection with the differentiation of neuroblasts and ganglion cells. Nevertheless, the striking agreement in the number and arrangement of the neuroblasts of *Xiphidium* and the "sense-organs" of the scorpion, a form in most respects so far removed from the Insecta, warrants the suggestion that the so-called sense-organs in the Arachnid are, like the circular spots in the procephalic lobes of *Xiphidium*, simply the expression of an early differentiation of the primitive ectoderm into

¹ *Vergleichende Studien*, etc. Denkschrift: d. k. Akad. d. Wiss. Wien, Bd. LVI, 1889, p. 48.

² Quart. Journ. Micr. Sci., XXXI, 1890.

³ Quart. Journ. Micr. Sci., Vol. XXXI, 1890.

integumentary cells (dermatoblasts) and nerve-cells (neuroblasts).¹

¹ Since making the above observations, my attention has been called to a recent article by Viallanes ("Sur quelques points de l'histoire du développement embryonnaire de la Mante religieuse" (*Mantis religiosa*), *Revue Biolog. du Nord de la France*, Tome II, No. 12, September, 1890). The description of the *Mantis* brain as given by this investigator agrees very closely with the results obtained from a study of *Xiphidium*. Reserving a consideration of the segmentation of the brain and optic ganglia for future publication, I here quote Viallanes' remarks on the development of the ganglia from neuroblasts ("cellules gangliogènes"): "Le premier lobe protocérébral constitué primitivement par une seule assise de grosses cellules (cellules gangliogènes), ne tarde pas à multiplier ses éléments. Les cellules gangliogènes entrent en division et produisent à leur face profonde de nouveaux éléments; ces derniers, se divisant eux-mêmes très rapidement donnent naissance à de petites cellules, pauvres en protoplasma, à noyau remarquablement chromatique, qui se multiplient elles-mêmes, et que nous pouvons dès maintenant désigner sous le nom de cellules nerveuses ou ganglionnaires. Grâce à cette prolifération, le premier lobe protocérébral, de simple assise cellulaire qu'il était au début, est devenu un massif cellulaire fortement convexe du côté de la plaque optique et formé maintenant de deux couches bien distinctes, l'une externe (limitant la face convexe du lobe), formée d'une seule assise de grandes cellules gangliogènes, l'autre interne, formée d'un puissant amas de petites cellules nerveuses."

CLARK UNIVERSITY, WORCESTER, MASS.,
January 15, 1891.

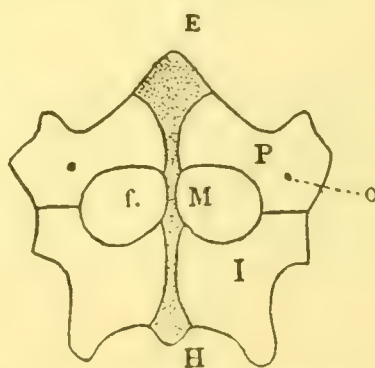
THE PELVIS OF THE TESTUDINATA, WITH NOTES ON THE EVOLUTION OF THE PELVIS IN GENERAL.

G. BAUR.

THAT *Sphenodon* is the most generalized living reptile there cannot be any doubt to-day. Let us take the pelvis of *Sphenodon* as a type of reptilian pelvis, and see in what relations the pelvis of the Testudinata and other vertebrates stands. The pelvis of *Sphenodon* consists of three ossified elements on each side, the ilium, pubis, and ischium, which all meet in the acetabulum. The ilium is a simple bone; pubis and ischium are more complicated, each one consisting of two branches. These conditions are best seen in the figure. The inner branch of the pubis may be called *entopubis*; the outer branch, *ectopubis* (pectineal process aut.); the inner branch of the ischium, *entoischium*; the outer, *ectoischium* (metischial process or tuberosity of the ischium, Huxley¹).

FIG. 1. — *Sphenodon punctatum*, Gray.

E, Epigastroid.
M, Mesogastroid.
H, Hypogastroid.
P, Pubis.
I, Ischium.
f, Foramen pubo-ischiadicum.
o, Foramen obturatorium.



The entopubes do not touch each other, nor do the entoischia; the entopubes are also separated from the entoischia. This separation is produced by a continuous rod of cartilage placed in the middle line. This cartilage may be called the *gastral cartilage*, *gastrale*, or *gastroid*. The part in front of the entopubis I call *epigastroid* (epipubis, part); the middle portion,

¹ Huxley, Professor: *On the Characters of the Pelvis in the Mammalia, and the Conclusions respecting the Origin of Mammals which may be based on them.* Proc. Roy. Soc., No. 194, 1879, p. 405.

mesogastroid; the portion behind the entoischia, the *hypogastroid* (hypoischium, os cloacæ). For the correct understanding of the pelvis of vertebrates it is necessary to introduce these terms. The foramina on each side between pelvis and ischium are called foramina pubo-ischiadica; the small foramen in the pubis, obturator foramen.¹

It is very easy to derive all conditions seen in the pelvis of the Testudinata from the condition of *Sphenodon* just described.

We start from the Chelydroids as a general type. In the young *Macrochelys* and *Chelydra* we have the gastral cartilage complete and well developed. The epigastroid forms a very

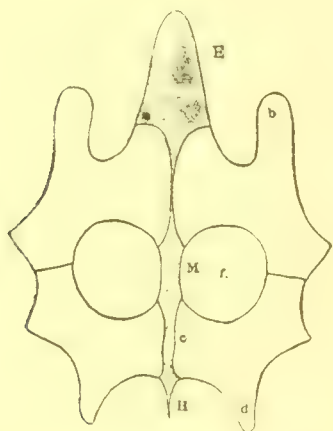


FIG. 2. — *Macrochelys Temminckii*
(Troost. MSS.).

- E*, Epigastroid.
- M*, Mesogastroid.
- H*, Hypogastroid.
- f*, For. pubo-isch.
- a*, Entopubis.
- b*, Ectopubis.
- c*, Entoischium.
- d*, Ectoischium.

massive, long, anterior process; the hypogastroid is very slender, ending in a point behind. In old specimens the entopubes touch each other in the middle line, separating epigastroid and mesogastroid; the entoischia also meet, separating mesogastroid and hypogastroid. Ossification may take place from different centres in the epigastroid, mesogastroid, and hypogastroid. Entopubes and entoischia never meet, but are always separated by the mesogastroid.

From the Chelydridæ we reach the conditions seen in the Cinosternidæ through the Dermatemydidæ and Staurotypidæ.²

¹ The obturator foramen may be placed completely in the pubis, or on the border of the pubis, or in the pubo-ischiadic foramen.

² I am now able — thanks to Professor Rüttimeyer — to give additional characters for the Chelydridæ, Dermatemydidæ, Staurotypidæ, based on the shoulder girdle, the pelvis, and the conditions of the ninth and tenth dorsal vertebra.

Chelydridæ. No anterior process on entoscapula near acetabulum; no posterior process on coracoid near acetabulum; mesogastroid well developed, separating completely entopubes and entoischia; no anterior process on ilium; rib-head of eighth

In the two families mentioned we have about the same arrangement as in the Chelydridæ. In adult Cinosternidæ we find the three gastroids ossified, but very small; the epigastroid never

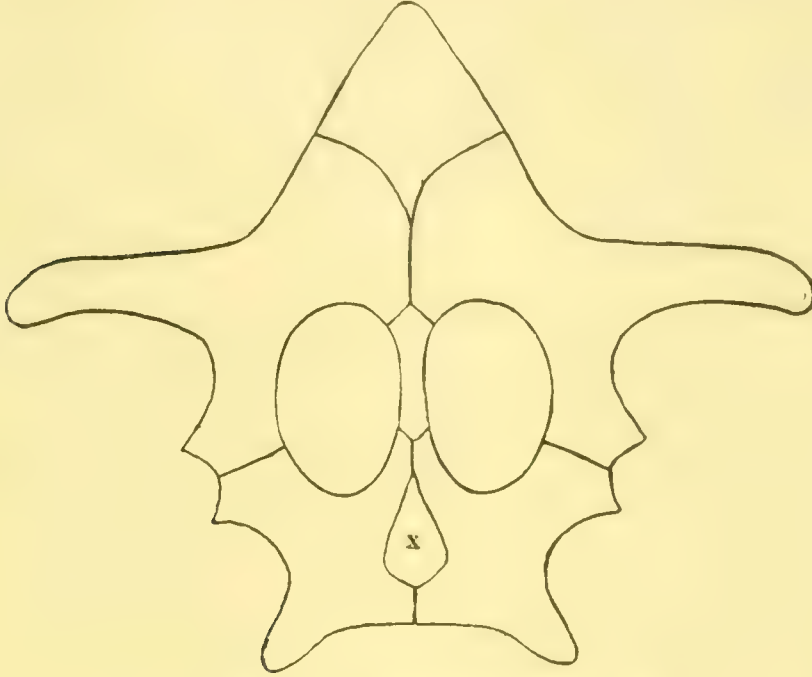


FIG. 3. — *Dermatemys Mawii*, Gray.

Pelvis from below, from a sketch of Professor Rütimeyer. X, peculiar ossified process, developed from the gastroid portion between the entoschia, also present in old specimens of Chelydridæ and Staurotypidæ.

reaches the extension seen in the Chelydridæ and Staurotypidæ; entopubes and entoschia nearly touch each other, being only separated by the small, diamond-shaped mesogastroid. It may be that in very old specimens the mesogastroid becomes absorbed by entopubes and entoschia.

pleurale well developed; an entoplastron; generally no rib on tenth dorsal; number of peripherals, 11.

Dermatemydidæ. An anterior process on entoscapula near acetabulum; a posterior process on coracoid near acetabulum; mesogastroid well developed, separating completely entopubes and entoschia; no anterior process on ilium; rib-head of eighth pleurale present; a rib on tenth dorsal which is free from the eighth pleurale; number of peripherals, 11; an entoplastron.

Staurotypidæ (Claudius, Staurotypus). An anterior process on entoscapula near acetabulum; a posterior process on coracoid near acetabulum; mesogastroid well developed, separating completely entopubes and entoschia; an anterior process on ilium; rib-head on eighth pleurale absent; no rib on tenth dorsal; number of peripherals, 10; an entoplastron.

Cinosternidæ. Like Staurotypidæ, but mesogastroid reduced, not separating entopubes and entoschia; no entoplastron.

In another direction, possibly from the Platysternidæ, developed the form of pelvis seen in the Emydidæ and Testudinidæ. Among the more generalized forms of Emydidæ, like *Malaco-*

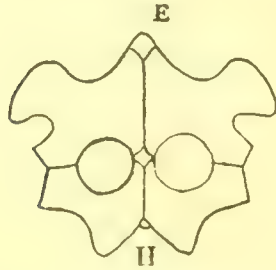


FIG. 4. — *Cinosternum pennsylvanicum*, var.

E, Epigastroid.

H, Hypogastroid.

clemmys, we find that entopubes and entoischia just begin to touch each other. In the young animals there is a continuous gastroid cartilage, only in older animals entopubes meet, and so do the entoischia. In this stage we find a cartilaginous epigastroid, mesogastroid, and hypogastroid. The same condition I have observed in *Chrysemys picta*. In the next stage entopubes and entoischia unite, but the cartilaginous mesogastroid is still present (Trachemys, Pseudemys, Terrapene, Clemmys, Geœmyda). In *Emys* the mesogastroid becomes ossified, and forms a slender element, pointed behind, and placed on the ventral side on the anterior portion of the united entoischia. *Cyclemys* is very near this stage. The epigastroid is always present in

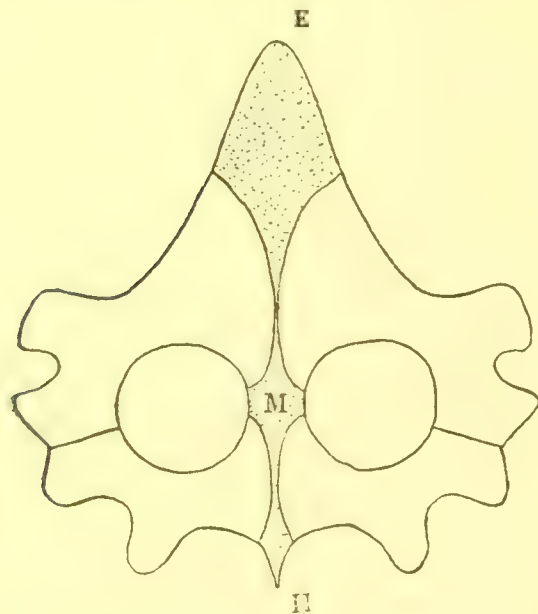


FIG. 5. — *Malacoclemmys geographica*, Les.

E, Epigastroid.

M, Mesogastroid.

H, Hypogastroid.

middle-aged specimens. It may calcify or ossify, or may become absorbed by the entopubes. The hypogastroid I never found ossified; it is either cartilaginous, but very small, or absorbed

by the entoischia. In the Testudinidæ, or true land tortoises, I have never seen an ossified gastroid. The whole gastroid cartilage may become absorbed by the pubes and ischia in adult animals, and it may happen that all elements co-ossify. This I have also seen in a very old specimen of *Clemmys guttata*. In half-grown Testudinidæ the gastroid cartilage is complete, but entopubes and entoischia are already united.

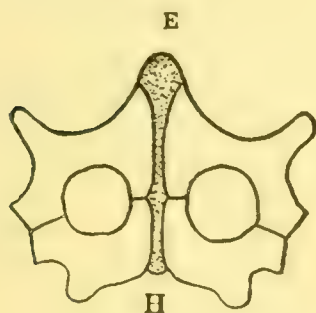
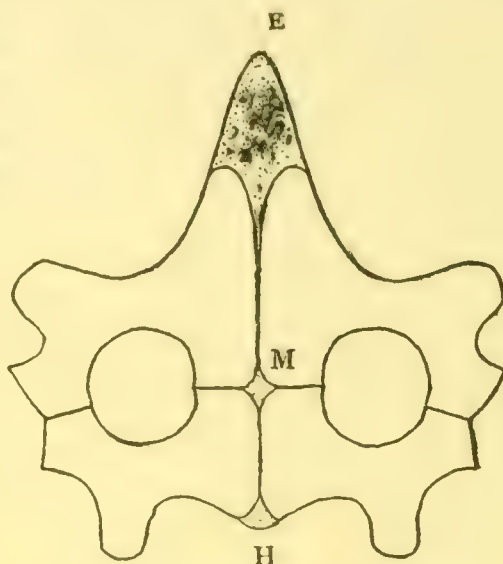


FIG. 6.

Testudo europæa, L.*E*, Epigastroid.*H*, Hypogastroid.FIG. 7. — *Trachemys elegans*, Wied.*E*, Epigastroid.*M*, Mesogastroid.*H*, Hypogastroid.

The pelvis of *Platysternum*, the only representative of the Platysternidæ, seems to be of the pattern of the Chelydridæ. I have no specimen at hand. Boulenger says: "The pelvis is intermediate between that of typical Emydoids, in which the pelvis and ischium are in contact on the median line, limiting two obturator foramina, and that of Chelydra, in which the two bones diverge, and are connected by ligament. In *Platysternum* the symphyseal branches of the pubis and ischium are parallel, but yet connected only by ligament. I must remark here that the former type of pelvis, *i.e.* with two obturator foramina separated by the union on the median line of the symphyseal branches of the pubis and ischium, occurs in all Testudinidæ (land and fresh-water) which I have examined, with the single exception of *Dermatemys*, which belongs in this respect to the Chelydroid type; also in the Cinosternidæ, but not in the Staurotypidæ, which belong to the latter type."¹

¹ Boulenger, G. A.: *Notes on the Osteology of the Genus Platysternum*. Ann. Mag. Nat. Hist., June, 1887, pp. 461-463, Pls. XVI, XVII.

From what I have said above, some of these statements have to be modified. I believe also that what is called ligament by Boulenger is really cartilage, and we would have, therefore, in *Platysternum*, a cartilaginous mesogastroid separating entopubes and entoischia. Boulenger does not mention the epigastroid and hypogastroid, but I conclude that these elements are also present.

The condition of the pelvis seen in the Pinnata can also be derived from that in the Chelydridæ. In the living Cheloniidæ ectopubes are far separated from the ectoischia, but are connected by the mesogastroid cartilage, which perhaps in very old specimens may become ligamentous. The epigastroid is present, rounded in front, and may become calcified or ossified in old specimens; the hypoischium is reduced entirely. Even in

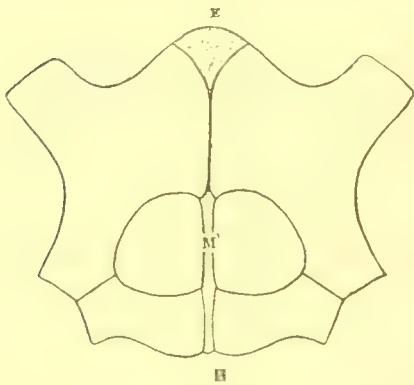


FIG. 8. — *Chelonia mydas*, L.

E, Epigastroid.

M, Mesogastroid.

H, Hypogastroid.

pretty large specimens (length of bony coracoid 255 mm.) the gastroid cartilage is still continuous. Between the entopubes it is visible from above, between the entoischia from below, being always placed on the sharp angle in which these elements meet. In some fossil Cheloniidæ, like *Allopleuron*, entopubes and entoischia are nearer together. This leads to the condition seen in the Dermochelyidæ, in which entopubes and entoischia seem to meet each other. I could not examine a fresh specimen of *Dermochelys*, and have to rely on Wagler's¹ and Hoffmann's² figures, Gervais'³ not being at hand. According to these figures the entopubes seem to touch the entoischia, but there is some uncertainty about it. In Hoffmann's figure the left entopubis reaches the ischium, but the right one does not.

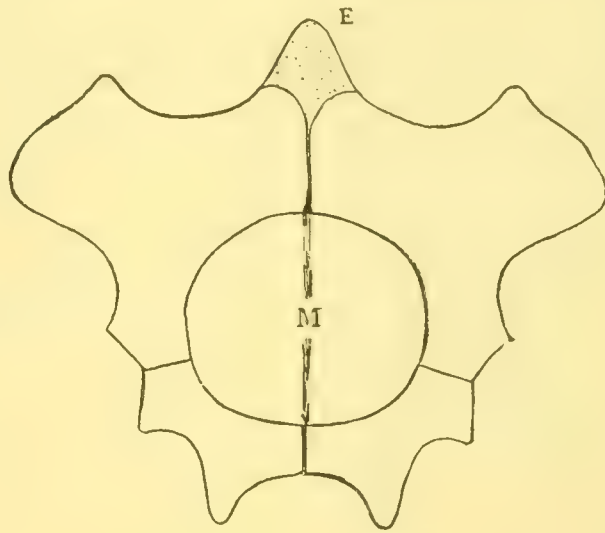
¹ Wagler, Joh.: *Natürliches System der Amphibien*, 1830, Pls. I, Figs. 21, 22.

² Hoffmann, C: *Reptilien*, in Bronn's Klassen und Ordnungen, Taf. XI, f. 3.

³ Gervais, P.: *Ostéologie du Sphargis Luth.*, Nouv. Arch. Mus. VIII. Paris, 1872.

In Wagler's figures the cartilages are not indicated. It is important to re-examine young and fresh specimens. The pubo-ischiadic foramina are very small; the mesogastroid seems to be greatly developed. The entopubes have a strong posterior branch, as in the Emydidae and Chelydridae, and also some of the fossil Cheloniidae, and meet in the middle line. The ischia are of the type of the Pinnata, and so is the ilium. Hoffmann figures a very strong epigastroid. It seems that Dermochelys developed from one of the more generalized Pinnata, which still possessed the posterior branch of the entopubis of the Chelydridae.

FIG. 9. — *Trionyx*.
E, Epigastroid.
M, Mesogastroid ligament.



In the Trionychidae we have a stage still more advanced than that seen in the Cheloniidae. The mesogastroid is only represented by ligament, and entopubes and entoischia are far separated; there is a well-developed cartilaginous epigastroid; the hypogastroid is exceedingly small. This condition must already have existed in the forms of the Laramie Cretaceous, in which the pelvis is not different from the living forms. That the Trionychian ancestors had a pelvis with a cartilaginous mesogastroid, and a pubis with a posterior entopubic process, there cannot be any doubt. I also believe that the young or embryonic Trionychidae will show the mesogastroid ligament represented by cartilage.

PLEURODIRA.

It was the peculiar condition seen in the pelvis of the Chelyiidae, which induced me to examine the pelvis of the Tes-

tudinata in fresh specimens. In *Chelys*, *Hydraspis*, and especially in *Emydura*,¹ the epigastroid is enormously developed, more than in any other form. As it is well known, the ectopubes and the branch connecting the entoischia with the ectoischia, are co-ossified with the plastron, the ilium with the carapace. In all the *Pleurodira* examined the epigastroid is united to the plastron by ligament: this ligament is attached on the posterior part of the suture between the hypoplastra. The epigastroid in *Chelodina*² is only about half as long as in the other *Chelyiidae*. In the *Podocnemididae* and *Sternotheriidae* it is well developed, but not so elongated as in the *Chelyiidae*.

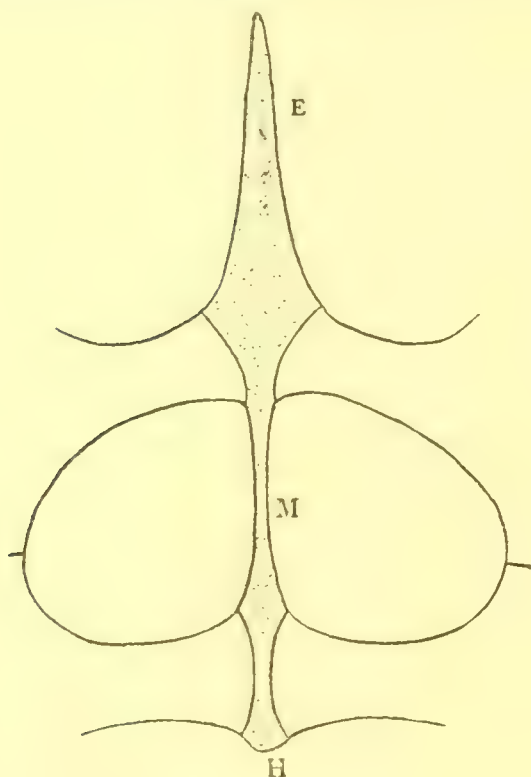


FIG. 10. — *Chelys fimbriata*, Schn.

Pelvis from above.

E, Epigastroid.

M, Mesogastroid.

H, Hypogastroid.

In young animals the gastroid cartilage is complete, but in very old animals entopubes are united, and also the entoischia. The hypogastroid is very small, or quite reduced; but the mesogastroid is always cartilaginous, forming a long element, separating entopubes and entoischia. In none of the *Pleurodira* the entopubes touch the entoischia. In all living *Pleurodira*, as far as I

¹ This fact was first seen by Hoffmann, who figured the long epigastroid in *Chelymys victoriæ*, in his *Reptilien*, Pl. VII, Fig. 6.

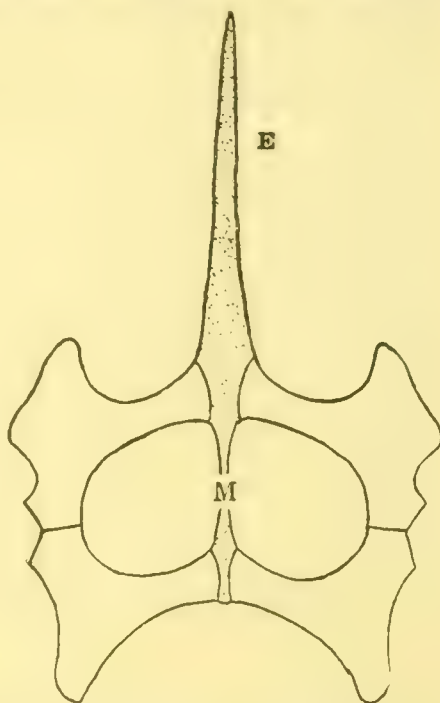
² I may mention here the peculiar fact, that in all the specimens of *Chelodina* examined by me there was only a single frontal, without any trace of a middle suture. This is the only exception among Tortoises, so far as I know.

know, the suture between the entopubes is short — in other words, the entopubes are slender; in the Cryptodira and Trionychia the entopubes are always much expanded. This slenderness of the entopubes, which is also present in the entoischia, seems to be produced by the co-ossification of ectopubes and ectoischia with the plastron, for which purpose material of the other elements has been used. In Compsemys of the Amphichelydia, and in Plesiochelys we have a similar condition of the pubis. In the Amphichelydia entopubes are widely separated from the entoischia. In Baëna we find the condition of Chelydra, but the tendency is for co-ossification of ectopubes and ectoischia with the plastron.

FIG. II. — *Emydura Krefftii*, Gray.

E, Epigastroid.

M, Mesogastroid.



It is now very easy to understand the evolution of the pelvis of the Testudinata. The oldest Testudinata possessed a pelvis very much like that seen in Sphenodon, but with the obturator foramen between pubes and ischium; the gastroid cartilage was continuous; epi- and hypogastroids were present, and the mesogastroid separated the entopubes and entoischia. This form was present in the Amphichelydia, and is still preserved in the Chelydridæ, Dermatemydidæ, Staurotypidæ and Platysternidæ. The entopubes and entoischia gradually approached and united, *a.* Cinosternidæ, *b.* Emydidæ, Testudinidæ; or became more separated from each other, until they were represented by ligament, *a.* Cheloniidæ,¹ *b.* Trionychia.

¹ As I stated above, the marine ancestors of the Cheloniidæ must have had a pelvis more or less like the Chelydridæ: from such a form the Dermochelyiidæ developed.

The ectopubes and entoschia remained separate, the posterior branch of the entopubes became reduced; ectopubes and entoschia co-ossified with plastron: Pleurodira.

The pelvis of *Sphenodon* not only explains the pelvis of the Testudinata, but also that of the Squamata and Ichthyosauria,¹ in which entopubes and entoschia become far separated.

The pelvis of the Aëtosauria, Belodontia, Megalosauria,² Cetiosauria,² is also easily referable to that of *Sphenodon*.

Iguanodontia² and Birds have become modified; in these forms the entopubes have taken a parallel direction to the entoschia. The ectopubis (pectinal process, præpubis) is more or less developed, being directed forwards and outwards, in the Agathaumidæ (Ceratopsidæ) the entopubes have become exceedingly reduced, the ectopubes greatly developed. In Birds the ectopubes are in a very rudimentary condition, and may be placed functionally on the ilia.

But *Sphenodon* does not offer the most original form of a pelvis in the higher vertebrates. The Proganosauria show conditions still more primitive. In Palæohatteria, for instance, we seem to have the gastroid cartilage very much more developed. The entire space between pubes and ischia seems to have been occupied by the gastroid cartilage. In *Mesosaurus*, an aquatic Proganosaurian, we have conditions resembling the Plesiosauria. In these forms the gastroid cartilage was probably interrupted, a distinct mesogastroid being present.

The pelvis of the Theromora can be explained by the condition seen in Palæohatteria. The ossification of pubes and ischia extended more and more, until the whole median portion of the gastroid cartilage was absorbed, only leaving a small obturator foramen. In this group pubes and ischia form a single broad plate, separated by a suture in which also the obturator foramen is placed. Still here we have the pubis turned forwards.

In Mammals the entopubes are turned backwards and united

¹ I may mention here that I have no doubt that the Ichthyosauria possessed, like the Rhynchocephalia, a small cartilaginous sternum. The whole morphology of the shoulder girdle strongly supports this opinion.

² I consider, with Seeley, the Dinosauria as an absolutely unnatural group, which is to be split into three distinct orders,—the Megalosauria, Cetiosauria, Iguanodontia, corresponding to the Dinosaurian orders Teropoda, Sauropoda, Orthopoda. The Dinosauria are comparable to the Enaliosauria, which contained also two entirely different groups, the Ichthyosauria and Plesiosauria.

to the entoischia, leaving a pubo-ischiatic foramen on each side. We have to take a form of pubis as seen in *Eryops*¹ or *Propap-pus*,² as related to the mammalian pelvis. I think it probable that a form of pelvis as seen in *Eryops*, but not so much ossified, was very near the original form of pelvis seen in Mammals. The pelvis of *Sphenodon* seems to be in the same relation to that of *Palæohatteria* as the pelvis of Mammals to that of an *Eryops*-like form, in which ossification was not so much advanced.

We come now to the Crocodilia and Pterosauria. The pelvis of these groups has always been a puzzle to the morphologist. As it is well known, we find here two elements in front, which do not take part in the formation of the acetabulum. There are two opinions: most of the authors declare these elements as the true pubes; others say the pubes are united with the ischia, and the elements in question correspond to the marsupial bones in Mammals, or the ypsiloid cartilages in Batrachia. Huxley is of the opinion that the bones are homologues of the marsupial bones in Pterodactyls, but that they represent true pubes in the Crocodiles.

Before discussing this question, it is necessary to consider the peculiar elements called marsupial bones, ypsiloid cartilages, epipubes. The question is, are these elements portions of the epigastroid cartilage, or are they developed independently from it? The oldest Batrachia, the Proteida, do not have these elements; they are well developed in *Menopoma*, *Megalobatrachus* and many of the Urodela. According to Wiedersheim, who has made very extensive ontogenetic researches on the pelvis of vertebrates, this portion develops very late: "Ganz zuletzt entsteht die Cartilago epipubis, und zwar in directem Zusammenhang mit dem allmählich auftretenden Symphysengewebe. Dieselbe stellt ein oralwärts gerichtetes, zapfenartiges und anfaenglich noch gänzlich ungegabeltes Gebilde dar." — *Anat. Anz.*, 1889, Nr. 14, p. 435.

In a former communication, Wiedersheim was in doubt about the homology of this element:³ "Über die morphol-

¹ Cope, E. D., *Amer. Nat.*, 1884, Pl. III.

² Lydekker, R., *Catal. of Foss. Rept. and Amph.*, Part IV, London, 1890, p. 120, Fig. 26.

³ Wiedersheim, R.: *Zur Urgeschichte des Beckens*. Ber. Naturf. Gesellsch., Freiburg i. B., Vol. IV.

ogische Bedeutung der sogenannten *Cartilago ypsiloides* oder *epipubis* halte ich mit meinem Urtheil vorderhand noch zurück."

In his latest paper on the subject (*Anat. Anz.*, 4. Jan., 1890), the *epipubis* is compared with the *episternum*: "Wie dem Schultergürtel vorn das *Episternum*, so sitzt dem Beckengürtel der Urodelen und weniger Anuren vorn die *Cartilago epipubis* auf. *Sternum*, *Episternum* und *Cartilago epipubis* der Amphibien sind homologe Bildungen, vorauf bereits Götte hingewiesen hat."

Wiedersheim is inclined — to conclude from his text-books — to consider these elements as the same as the *epigastroid*. I do not believe that this is the case. The *epigastroid* is always, also, in *Necturus*,¹ in which I could study its evolution on material kindly given me by Professor C. O. Whitman, the anterior portion of the *gastroid* cartilage, from which it is not developed independently. I believe that the *ypsiloid* cartilages are of secondary origin, developing independently from the *gastroid* cartilage. The long *epigastroid* of the *Chelyiidae* is homologue to the short *epigastroid* in *Testudinidae*; homologue to the anterior portion of the *gastroid* cartilage in *Necturus*; homologue to that portion of the *gastroid* cartilage in *Salamanders* and *Dactyletra*, to which the *ypsiloid* cartilages are connected. I consider these cartilages as a later acquisition; they may develop in any group, — *Batrachia*, *Pterosauria*, *Monotremata*, *Marsupialia*. The question now is, how to name these elements. The name *epipubis* is not good, having been used, also, for an element which is not homologue. *Ypsiloid* cartilages and *marsupial* bones are also inappropriate names. I think it best to introduce the name *cartilago pyramidalis*, to express the relation of these elements to the *musculus pyramidalis*.

We have to return now to the *Crocodylia* and *Pterosauria*. The peculiar condition of the pelvis of the *Crocodylia* exists already in the *Lias*. This structure, therefore, is a very old one, and I have some doubt whether embryology will give us any help in this question. Perhaps it is possible to get some light by examining the pelvis of the *Pterosauria*. I was always inclined to consider the *ischium* of *Crocodylia* and *Pterosauria* as this element alone, and the anterior bones as the true *pubes*;

¹ I may state here that in *Necturus* a distinct but very small *sternum* is present. I found it in all specimens examined.

but I think now that this opinion is not correct, and that we have to adopt the view of Leydig, Fürbringer, and Seeley, that the ischium contains also the pubis, and that the free elements in front are not the pubes. If we study the ischium of a *Pterodactyl*, we find that it forms a very broad plate, which is firmly co-ossified with the ilium; between these two bones the acetabulum is placed. In the ischium we find, according to Zittel (*Handbuch der Palaeontologie*, Vol. III, p. 786), a small foramen below the acetabulum in *Rhamphorhynchus* and *Dimorphodon*. This foramen is said to be very much larger in *Pterodactylus antiquus*. In other species the "ischium" is divided by the extension of this foramen into an anterior and posterior branch. The bones called pubes are entirely excluded from the acetabulum, and are connected with the anterior portion of the "ischium." They are either separated from each other, expanded distally, or united and slender, having on each side a lateral process. I think that this condition can only be explained in this way: The foramen in the broad "ischium" represents the obturator foramen; the pubis must therefore be united with the ischium, of which it forms the anterior portion; the elements excluded from the acetabulum and connected with the distal anterior end of the pubic portion of the ischium represent the cartilagines pyramidales.

From this standpoint I think we have to look also at the pelvis of the Crocodilia. It seems to be more probable to consider the bone generally called ischium as the united pubis and ischium; the so-called pubis as the cartilago pyramidalis. Palæontology has to decide this question. It is possible that the triassic ancestors of the Crocodilia (the Aëtosauria and Belodontia do not belong here) will bring some light. In some of the Dicynodonts we find the pubis greatly reduced, and it is not unlikely that the pelvis of the ancestors of the Crocodilia will show some resemblance to such a form. By this of course I do not wish to express that the Dicynodonts are in any way related to the Crocodiles.

After having gone over the different groups of higher vertebrates, we may now examine the history of the pelvis in the lower types. Here I have little to add to the ontogenetic researches of Wiedersheim, with whom I entirely agree in his general results about the origin of the pelvis, which I can con-

firm after the study of the evolution of the pelvis in the most primitive Batrachian *Necturus*.

From a form of pelvis as seen in *Palæohatteria* it is only one step to the Batrachian pelvis; for instance, *Necturus*. Here the gastroid cartilage is greatly developed, pierced only by the small obturator foramen; only the ischia are ossified; the pubes are not distinct from the gastroid cartilage. One step lower, and we have the pelvis of the *Dipnoa* or *Chlamydoselachus*, only represented by the gastroid cartilage.

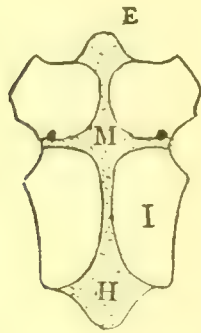


FIG. 12. — *Palæohatteria*, Credner.

E, Epigastroid.
M, Mesogastroid.
H, Hypogastroid.
I, Ischium.



FIG. 13. — *Necturus maculatus*, Raf.

E, Epigastroid.
M, Mesogastroid.
H, Hypogastroid.
I, Ischium.

Wiedersheim has expressed the opinion that the single median gastroid cartilage ("unpaare ventrale Beckenplatte") in the *Dipnoa* takes its origin from two halves. The pelvis of *Necturus* is nearest to the pelvis of the *Dipnoa*, and in this form the gastroid cartilage develops from two halves. In a specimen 25 mm. long the gastroid cartilage is represented by two lateral portions; in a little older stage the two halves are united, remain united, and extend now forwards, forming the long epigastroid portion.¹

There is one element in the pelvis which has to be mentioned

¹ I may mention here the fact that *Necturus*, like *Proteus* (Wiedersheim), develops its limbs like all the other Urodela examined, by budding. There is never an indication of more than four digits. This seems to be another proof for my theory that the limbs of the Stapedifera have developed by budding, and that the ancestors of the Stapedifera were not, as it is generally believed, polydactyle forms. Baur, G.: *Beiträge zur Morphologie des Carpus und Tarsus*, Jena, 1888.

—the acetabular bone of the Mammalia.¹ This element, I think, originated during the evolution of Mammalia, and may appear in any group.

Mehnert² has expressed the opinion that a three-rayed pelvis is the original form for all Amniota. This seems to be correct, with the exception, perhaps, of the oldest Amniota, which seem to have had a continuous gastral cartilage, as in *Necturus* for instance, in which pubis and ischium became ossified. The history of the vertebrate pelvis seems to be this:—

1. Continuous gastral cartilage, extending between the femora. Dipnoa, Selachia part.

2. Continuous gastral cartilage, in which the ischium developed as a separate ossification. Proteida.

3. Continuous gastral cartilage, in which pubis and ischium appeared as separate ossifications. Batrachia part, Proganosauria part.

4. *a.* Pubic and ischiadic ossifications, extending over whole gastral cartilage, Theromora, permian Batrachia part. Crocodilia, Pterosauria (?). *b.* Gastral cartilage between pubis and ischium disappearing; appearance of foramina pubo-ischiadica; all other Amniota.

¹ In the sketch of the pelvis of *Dermatemys* sent me by Professor Rütmeier a small acetabular bone is present.

² Mehnert, E.: *Untersuchungen über die Entwicklung des Beckengüstels bei einigen Säugethieren*. Morph. Jahrb., XV, p. 111, also Morph., Jahrb. XIII. Mehnert's latest publication treats about the development of the pelvis in *Emys orbicularis*, L. (Morph. Jahrb., Vol. XVI, Part 4). The account of the phylogeny of the pelvis of the Testudinata given in this paper will modify some of the opinions expressed by Dr. Mehnert.

SPERMATOPHORES AS A MEANS OF HYPO- DERMIC IMPREGNATION.

C. O. WHITMAN.

ALTHOUGH it is well known that spermatophores are of very general occurrence among the invertebrates, and even among many vertebrates, the assertion that, as a perfectly regular and normal affair, in animals as highly organized as the leeches, *they represent an injecting apparatus, by means of which the spermatic elements of one individual are forced through the body-wall of another, at any point whatsoever*, may appear almost incredible, even when supported by direct observation many times repeated on different species. That such is certainly the case, however, is very easily demonstrated, and any one can verify it as often as he likes on almost any species of Clepsine that happens to be accessible. The observations to be presented in this paper will make this fact abundantly evident.

But what becomes of these spermatic injections? Do they ever reach the eggs and fertilize them? and if so, is this the normal method of bringing the sexual products together? Although I cannot affirm this as a positive certainty, the evidence seems to me to fall but little short of being conclusive. I have studied closely the habits of these leeches in Europe, Japan, and America, and with especial reference to settling the question of when and how impregnation is effected. Long-continued observation under most favorable circumstances has never given me so much as a single indication that the genital pores are ever united in the act of copulation. On the other hand, the planting of spermatophores on the surface of the body at any point that happens to come first, is a common occurrence, which one may often see repeated several times in the course of a few hours by the same individual. I have followed the track of the spermatozoa from the point of penetration to the coelomic cavity in which the ovaries lie, but I have not pursued the subject far enough to determine when or how the spermatozoa pass through the wall of the ovisacs. That spermatozoa get

into the ovisacs, and that fertilization takes place before oviposition, can be demonstrated by facts that admit of no doubt. The passage through the wall of the ovisac—the only link in the chain of direct evidence yet to be supplied—seems to be an inference justified by all the known facts. Such a passage, in the absence of any definite openings in the ovarian walls, would have to be a forced one, depending upon the action of the spermatozoa themselves. As these walls are represented by a thin membrane which becomes enormously distended as the eggs enlarge to maturity, the difficulty of penetration could not be great; and the case of *Peripatus* and the Turbellarians seems to show that spermatozoa are capable of effecting automatically such a passage.

The view here taken finds a very strong confirmation in the fact that precisely the same mode of copulation occurs in many Turbellarians, in the Rotifers, and in *Dinophilus*, as the citations from Lang, Plate, and Harmer, given farther on, fully show. The indications are that it occurs also in many oligochaetous annelids, as well as in several genera of leeches besides *Clepsine*,—perhaps in all the *Rhynchobdellidæ*. The occurrence of such a mode of copulation among so many of the lower bilateral animals appears not only to render explicable the pluri-penial condition of many Turbellarians and some of the higher worms, but also to clear up many puzzling observations in regard to fertilization in animals that have no intromittent organ. It is no longer necessary to suppose that the spermatophores found attached to different parts of the body of *Peripatus* must be carried through the vagina and up the uteri in order to reach the eggs; and the discovery of spermatozoa projecting through the ovarian walls of this animal, as reported by Moseley and Sedgwick, ceases to be so complete a mystery. The difficulties in the way of understanding how spermatophores can be of any use when attached at a considerable distance from any genital pore, and completely closed externally, as described by Vejdovsky for many annelids, may not be so great as they have hitherto appeared.

The facts and bibliographical notes to be presented in this paper are sufficient, I think, to make it at least probable that the original function of the spermatophore was precisely what it now is in the Turbellarians, the Rotifers, *Dinophilus*, and

Clepsine, — *the injection of spermatozoa through the body-wall, or hypodermic impregnation*, as we may call it.

This mode of impregnation represents an important economical step in advance of the more primitive mode of setting the seminal elements free in the water. The deposition of sperm-capsules at random on any point of the external surface that happens to be accessible at the moment of meeting, is improved upon by restricting the act to a definite region, as one or more segments of the clitellum in certain annelids, or the surface around the external openings of the oviducts, as in the crayfish; and, still further, by limitation to the edge of genital pores or seminal receptacles, as in the copepods. The seminal reservoir of the lobster, discovered by Bumpus,¹ marks an advance on the conditions obtaining in the crayfish.

The habit of discharging spermatophores directly into the vaginal orifice, presupposing direct union of the sexual pores, brings us to relations where such copulatory organs as we find in the Gnathobdellidæ would become useful. The penis is here only an eversible end-piece of the *vasa deferentia* — a simple tubular elongation of what in its simplest form would be represented by a pore.

Lang, who was the first to discover this mode of impregnation in the Turbellaria, has thrown out the interesting suggestion, that in these animals the penes may have been primarily organs of attack and defence, which assumed secondarily the office of copulatory organs. The grounds for the suggestion may be seen from a citation to be introduced farther on. Such a mode of origin, though it may be true for the Turbellaria, does not invalidate the suggestion I have made for the Gnathobdellidæ, except on the supposition that the penes represent homologous organs in the two groups. Such a supposition appears to be forbidden by the absence of these organs in the lower leeches. It seems to me, therefore, altogether more probable that in the higher leeches they have been independently acquired, and that their evolution began after, or simultaneously with, the establishment of the habit of true copulation.

The structural and ontogenetic resemblances of the penes in the two groups cannot be taken as decisive proof of genetic identity. The resemblances between the penis and the pharynx

¹ *The Embryology of the American Lobster.* Journ. Morph., Vol. V. [In press.]

of a Turbellarian are of the same nature and equally close; but no one would in this case be likely to mistake such resemblances for homologies.¹

In view of the fact that the spermatophores of Clepsine were discovered nearly half a century ago by Friedrich Müller, and described as a "*phaenomenon cujus neque analogon inter reliqua animalia reperire*," and that they have since been observed by Max Schultze, Leuckart, Leydig, and Schneider, it may appear a little remarkable that their function should have so long escaped detection. But when we consider how totally unprepared were the minds of investigators for such a mode of impregnation, we find no difficulty in understanding how it comes to pass that the old belief still prevails, that all the Hirudinea, penis or no penis, copulate in essentially the same manner, and fecundate by conveying the spermatoc fluid of one individual (or of both reciprocally) directly into the female genital orifice of the other, thus placing it where it can pass unobstructed into the so-called uterus, or, in the absence of such a specialized part, into the ovarian sacs.

While this has been, and still is, the opinion generally received, the possibilities of the eggs being fertilized after deposit, of self-fertilization, and of parthenogenetic development, have not been overlooked. The following notes and extracts are designed to give the history of the subject, and to show what questions have been left unsettled.

HISTORICAL NOTES AND EXTRACTS RELATING TO THE HIRUDINEA.

1. *Clepsine complanata*.

FRIDERICUS MUELLER. De Hirudinibus circa Berolinum hucusque observatis. Berolini, 1844. pp. 33, 34.

"Cleps. complanatas, quamvis plurimas amoris tempore continua attentione observaverim, *coëuntes nunquam observavi*;"² sed eodem

¹ We are continually reminded that *parallel development* has been a much more important factor in evolution than has generally been supposed. Similar bases, similar needs, similar variations (because predetermined by like causes), guided by parallel selective influences, which would be sustained by like environments, have unquestionably resulted in numberless analogies which are usually allowed to pass as evidences of genetic affinity.

² Braun (*Systematische Beschreibung einiger Egelarten*, Berlin, 1805, p. 60) makes the same observation.

fere ante ovorum partum tempore, quo Cleps. tessulatae coire solent, singulare mihi in Cleps. complanatis sese obtulit phaenomenon, *cujus neque analogon inter reliqua animalia reperire*,¹ neque explicationem dare valeo. Ad utrumque nimirum faciei ventralis latus *organa singularia filiformia*, tres usque quinque corporis annulos longitudine aequantia modo simplicia, modo ad basin usque bipartita exseruntur, modo singula modo plura, modo in anteriore modo in posteriore corporis parte. Haec per plures dies propendent, dum animal, alioquin segnissimum, multo alacrius in vitro suo circumvagatur. Simul substantiae floccosae albae magna copia secernitur, totam mox vasis in quo servantur aquam turbidam reddens.

“*Inter phaenomenon hoc et propagationem relationem quandam existere, nullus dubito*; plurimas enim Cleps. complanatas per tria semestria domu observavi, neque vero alio unquam tempore *organa haec filiformia* eas exserere vidi, dum amoris tempore ne una quidem inter triginta et plures non exserebat. Praeterea his organis exsertis, ut in Cleps. tessulata post coitum, ovariorum motus peristalticus, quo ova a funiculis suis solvuntur, incipit. *Quo vero munere fungantur haec organa, nescio*; anatomica quoque disquisitione nihil de eo docente. Nam corpora quidem in crura dua reflexa divisa in tertio quovis annulo utrinque sub tractus intestinalis appendicibus latentes reperi, quibus replicatis organa illa filiformia fortassis formantur; num autem cum testiculis, quibus interjacent, aliave apparatus sexualis parte cohaereant, videre haud contigit.

“Corpora similia etiam in *C. verrucata, marginata, tessulata*, inveni, quamvis in nulla praeter complanatam specie organa filiformia exseri vidi.”

MAX SCHULTZE. Zoologische Skizzen. *Zeit. f. w. Zool.* IV. 1853. pp. 186, 187.

“Höchst auffallend ist, dass bei *Planaria torva* der Same in festen, retortenförmigen Spermatophoren verpackt übergeführt wird, welche man ein oder zwei an der Zahl nach der Begattung in dem beschriebenen Raume [recept. sem.] findet. Die aus einer braunen, chitinartigen Hülle bestehenden Spermatophoren platzen später, und fallen nach Entleerung des Inhaltes ganz zusammen. In diesem Zustande kann man sie im ersten Frühjahr bei fast jedem Individuum dieser Species sehen. *Ich erinnere hier an die Beobachtungen von Fr. Müller (Zei-*

¹ Nisi forte appendiculae generatrices a Morrenio (*De Lumbr. terrestr.* p. 77) sic dicta, quas in Lumbrico terrestr. auctor laudatus, in aliis pluribus Lumbricinis Cel. Dr. Hoffmeister et ipse observavimus, Cleps. complanatae organis filiformibus analogae.

tung für Zoologie etc. von D'Alton und Burmeister, No. 25, Juli, 1849), welche ich selbst bestätigen kann, dass bei Clepsine complanata und wahrscheinlich bei vielen Regenwürmern die Begattung durch Spermatophoren vermittelt wird."

A very important observation was made by Filippi, and confirmed by Grube; namely, that individuals isolated for some days before oviposition produced fertile eggs. This fact and the absence of a penis suggested self-fertilization (Filippi), or possibly an early internal fertilization (Grube).

F. DE FILIPPI. Lettera sopra l' Anatomia e lo Sviluppo delle Clepsine. 1839. p. 15.

"Tutti i zoologi si accordano nel dire che le sanguisughe sono ermafrodite; il che è vero anche per riguardo alle Clepsine; ma in queste gli organi de' due sessi sono totalmente diversi che negli altri generi della famiglia. È anche ammesso da tutti che le sanguisughe non possono fecondarsi da se, ma hanno bisogno del reciproco congiungimento di due individui; e qui mi occorre di far rimarcare un' eccezione che ci presentano le Clepsine. Infatti per le condizioni speciali de' loro organi generativi non può nemmeno aver luogo in esse una fecondazione interiore; *al che si aggiunga aver io l' esempio di un individuo, il quale mantenuto isolato in un vaso di cristallo nella mia camera partorì uova che in seguito si svilupparono.*"

ADOLPH EDUARD GRUBE. Untersuchungen ueber die Entwicklung der Clepsinen. Königsberg, 1844. p. 11.

"Gegen die Vermuthung, dass sich die Clepsinen äusserlich selbst befruchten, spricht der Umstand, dass ich an den frischgelegten Dotterkugeln oder in der Eiflüssigkeit nie Spermatozoen gefunden, was doch, wenn die Samenflüssigkeit mit den Dottern zugleich ausgeschüttet würde, kaum anders sein könnte, gegen die Annahme in's Besondere, dass sie sich äusserlich gegenseitig befruchten, der Beweis, dass wenn ein mehrere Tage abgesperrtes Individuum Eier legte, diese sämmtlich zur Entwicklung kamen. Vielleicht also erfolgt die Begattung doch innerlich, aber zu einer frühern Zeit? In diesem Fall müsste sie wenigstens 11 Tage vor dem Eierlegen eintreten, denn so lange hatte ich eine Clepsine abgesondert, deren Eier sich vollständig entwickelten, und die Enden der Samenleiter müssten dann sich umstülpen und als Ruthen dienen, oder die Spermatozoen gelangen in besondere Behälter geschlossen in die weiblichen Genitalien."

RUDOLF LEUCKART. Die Menschlichen Parasiten. I. 1863. pp. 675-680.

The place and mode of origin, and the form of the spermatophore were long ago correctly described by Leuckart. Speaking of the terminal double sac of the male organs of the Rhynchobdellidæ, he says :—

“ Der körnige Inhalt derselben dient zur Umhüllung des Samens und formt denselben im Innern des Begattungsapparates zu einer gleichfalls zweihörnigen *plumpen Spermatophore*, die bei der Begattung in die weibliche Oeffnung eingeschoben wird.” [pp. 675, 676.]

Leuckart assumes that in such forms as *Clepsine* there is direct copulation,—that is, by union of the sexual orifices,—and further, that fertilization is reciprocal (p. 673). But Leuckart has also seen spermatophores attached to the external pore of the female organs ; for he says :—

“ *Wo eine eigentliche Scheide fehlt (Bei den Rüsselegeln), da wird die Spermatophore in der weiblichen Oeffnung festgeklebt. Man sieht sie hier noch halbe Tage lang nach der Begattung ansitzen, bis die Spermatozoen in den Eierstocksschlauch übergetrieben sind. Auch bei den Arten mit Scheide findet man die Samenfäden später im Innern der Eierstock.*” [p. 680.]

2. *Clepsine tessulata*.

F. MÜLLER. Über die Geschlechtstheile von *Clepsine* und *Nephelis*. Müller's *Archiv*. 1846. p. 145.

“ Mit dem Fusse festsitzend saugt jedes der beiden Individuen mit dem Kopf sich an der Bauchseite des andern fest, worauf *ein konisches Organ aus der vordern Geschlechtsöffnung sich ausstülpt und in die hintere des anderen Thieres eintritt*; so vereinigt sitzen die Thiere meist mehrere Tage lang.”

C. tessulata, if Müller's observation be correct, takes an exceptional position, which is all the more difficult to explain, as *C. marginata*, and a number of other closely allied species found in America and Japan, certainly all agree in the habit of attaching their spermatophores to the exterior.

3. *Clepsine* var. *Porte-chaîne*.

Moq.-Tand. 1846. Pl. xiv. Fig. 5.

ÉBRARD. Nouvelle Monographie des Sangsues Médicinales. Paris, 1857.

“Aux derniers jours du mois de mai, en 1854, j’ai trouvé deux glossiphonies (variété dite *Porte-chaîne*) qui étaient accouplées. Elles étaient fixées, tête à tête, à la face inférieure d’une pierre par leurs ventouses qui étaient très-rapprochées. Leur corps était contourné de telle sorte qu’un de ses côtés touchait la pierre, et que l’autre était libre; *elles étaient accolées par la surface abdominale*. Une seule de ces annélides fécondait l’autre; car, les ayant séparées, je n’aperçus qu’une verge. Les ovaires de l’une d’elles se gonflèrent et se colorèrent peu à peu en blanc, et *quarante-cinq jours après elle fit des œufs*.” [pp. 60, 61.]

Ébrard had the question of reciprocal fecundation in mind, and entirely overlooked the spermatophores.

4. *Clepsine marginata*.C. O. WHITMAN. The Embryology of *Clepsine*. 1878. pp. 8, 9.

“I have found that eggs taken from the ovary at the time they are about to be laid develop in the normal manner, and have taken advantage of this to watch the earliest changes in the ripe egg. I have done this many times, and always with success. I regard this as very strong evidence that *impregnation takes place while the eggs are in the ovaries*. This is in harmony with the fact that I have found spermatozoa in the ovary two or three days before the time for depositing the eggs. It is barely possible that these spermatozoa found their way into the ovary accidentally during the dissecting. I can only say that no testicular sacs were ruptured during the process; but the *vasa deferentia* may have been severed, as they are so minute that one cannot easily see them. *The unchanged condition of the germinal vesicle at the time the eggs have attained their full size renders it probable that fecundation does not take place more than four or five days at the longest before the deposit*; but this does not prove that copulation may not have taken place at a much earlier date. *I isolated an individual which had just sucked itself full of blood, and which showed no signs of eggs through the body-wall, and after fifteen days obtained eggs that developed in the usual manner*. Recalling the fact that the growth of the egg from the primary egg-cell requires only twelve to fifteen days, it appears that this specimen was isolated about, or just before, the time when the egg-cell began to grow. *In another case eggs were obtained at the end of twelve days, which developed in the normal way*.

"These facts raise a suspicion that Clepsine is capable of self-fecundation. The question as to whether copulation occurs will be most satisfactorily settled by isolating young individuals and keeping them until they produce eggs.

May 2, 1878. — "Five individuals were isolated in the summer of 1877, at the time of hatching. Each has been kept in a separate vessel from that time to the present. Eggs were laid by one April 24th (this year), and hatched May 1st; by two others, April 29th. The latter are now in the germ-band stage. The eggs had in each case passed the pronuclear stage, at the time they were first noticed, so that I was unable to demonstrate by section the existence of a male pronucleus. As the eggs developed in the normal manner, it is very probable that they were fecundated. Here is an unquestionable case of *self-fructification*, or of *parthenogenesis* — more probably the former."

The above statements not only confirm the observation of Filippi and Grube, as to isolated individuals producing fertile eggs, but they also make it probable that fertilization is internal. To the evidence given by eggs taken artificially from the ovaries, I can now add another which seems to be perfectly conclusive. I have succeeded in finding a perfectly distinct and indubitable male pronucleus in the ripe ovarian egg of *C. marginata*.

What I formerly regarded as positive proof, either of self-fructification or of parthenogenesis, in the light of what I now know about the use of spermatophores, is open to some doubt. At the time of my experiment of rearing individuals from the egg in isolation, the possibility of hypodermic impregnation never crossed my mind, and I can now see where my observation was not sufficiently guarded to remove all doubt. In order to bring the five individuals to maturity, I had to feed them some ten or twelve times. I allowed them to take their meals from the same fish, only thinking it necessary to watch them from beginning to end, in order to see that no copulation took place. Whether I ever allowed them to come in contact long enough to deposit spermatophores, my notes do not show, and here is where the doubt comes in. The experiment ought to be repeated under conditions that would exclude every possibility of contact, and *C. marginata* would be one of the best species for such a purpose, as it is so easily reared. It would be well to isolate a large number of individuals, so as to have material

enough to allow of taking the ripe eggs from the ovaries in a few cases. The demonstration of the male pronucleus in such eggs would show that the leech is able to fertilize itself internally. If the male pronucleus were not found before oviposition, and should be found some time after it, one could infer external self-fecundation. If the male pronucleus proved to be wanting in both cases, we should have conclusive evidence of parthenogenesis, provided a considerable number of tests all gave like results, and provided, further, that the rest of the eggs developed embryos.

The importance of the experiment will be readily seen; for should self-fertilization be clearly proved under the conditions named, I think that fact, in the light of what we know about the breeding habits, would be sufficient to make it extremely probable that self-fertilization is a normal affair. That view would compel us to look upon the spermatophores attached to the surface as having nothing to do with fertilization. While self-fertilization certainly seems very improbable, we are not to forget that it is a possibility. It is believed to take place in Cestodes, and perhaps also in some Trematodes and Turbellaria. V. Baer (*Müll. Arch.*, 1835, p. 224) long ago reported a case in *Limnæus auricularis*, and Oken (*Isis*, 1817, p. 320) obtained fertile eggs from an individual of the same species reared in isolation.

5. *Nephelis*.

ISAO IIJIMA. Origin and Growth of the Eggs and Egg-strings in *Nephelis*. *Quart. Jour. Micr. Sci.* N. S. LXXXVI. April, 1882. pp. 196-197.

"The anterior portions of two individuals, attached by their suckers to the glass vessel near each other, are spirally entwined in such a manner that the ventral surfaces of their genital bands are always brought into apposition. They maintain this position for a considerable time, now and then changing the direction of their winding, and relaxing or tightening their hold. Sometimes the act is of short duration, and two or three times renewed at short intervals. At other times, and when disturbed, the act ceases altogether, or else they combine with other individuals. It is evident that there can be no reciprocal fecundation while the leeches are coupling in the position above described.

"*As there is no intromittent organ, it is probable that the male orifice with its prominent muscular lips clasps the female orifice, while the spermatozoa are forced onward by the action of the ejaculatory organ.*

"I am unable to say precisely at what time of the year copulation begins; but I found spermatozoa in the ovaries of one leech on the 20th of February, for the first time in this year. The act of coupling, so far as my experience goes, takes place almost always in the morning.

"**ABNORMAL COPULATION.** — *I have often found individuals with a small, two-horned, whitish body adhering to some portion of the genital band. The position of this cornuous body was always on or near the genital band, sometimes on the dorsal surface, sometimes on the extreme margin, but more frequently on the ventral surface than elsewhere. It consisted of two thin-walled bottle-shaped tubes (ca. 5 mm. long), the broader ends of which were inserted, close to each other, into a small disc-like portion. This portion, the margin of which presented a villiform appearance, was partially embedded in the epidermis. Around the disc was a discolored area, which proved to be, on examination of sections, a macerated portion of the epidermis. The two bottle-shaped tubes were filled with spermatozoa, and opened by means of two distinct holes in the disc. From each of these openings a stream of spermatozoa was found, penetrating to a considerable depth into the underlying tissues. In section the substance of the two-horned body appeared dotted and longitudinally striated, but I was unable to recognize any cellular structure.*

"For a long time I was much puzzled as to the meaning of all this, but from further observation was led to regard it as *a case of abnormal or unsuccessful copulation.*

"When disturbed during the act of copulation, the two leeches usually separate immediately; but *in one instance they did not separate even after putting them into chromic acid. On examination I found that the female orifice of each leech was not in contact with the male orifice of the other, but that each individual was attached to the ventral surface of the other by its male orifice, the female orifice remaining free. On separating them by force, each male orifice left the two-horned object on the body of the other.*

"*In another instance that came under my notice, only one individual had already deposited the two-horned body, while the other had a mass of spermatozoa hanging from its male orifice. The latter was dissected, and the two-horned body found occupying the whole interior of the ejaculatory organ, with the cavity of which it exactly corresponded in shape. It came out without any resistance. It thus became evident that the two-horned body belongs to the interior of the ejaculatory organ. That it forms no permanent part of the male organ seems evident, from the fact that sections made in the winter show no trace of such a body. I have not thus far been able to determine whether this body forms in the case of normal copulation also. As to its mode of*

formation, I have nothing to offer except the conjecture that it may be the hardened secretion of some of the glands of the ejaculatory organ.

"On many leeches were found scars, which very likely may have been the marks left by these peculiar bodies.

"It is hardly to be doubted that the normal mode of charging the ovaries with spermatophores is through the female orifice. It would certainly be impossible for the spermatozoa to find their way into the ovaries in many of those cases which we have described as abnormal, especially where the injection takes place far in front of the male orifice."

Professor Iijima's observations were made under my direction, and at the time I certainly concurred with him in his conclusions. Professor Iijima's incredulity was wholly due, as one may readily see from his own words, to the conviction that there was only one way provided by nature whereby the spermatozoa could reach the eggs within the ovaries; namely, through the female genital pore. The whole description tallies so closely with what I have seen in *Clepsine*, that I feel confident that the spermatophores serve the same end in both genera.

Schneider's observations on *Nephelis* are much less complete than Iijima's, and spermatophores seem to have entirely escaped him.

ANTON SCHNEIDER. Das Ei und seine Befruchtung. 1883. pp. 22, 32, 65.

"Die Begattung beginnt damit, dass sich die Thiere umeinander winden. Ihre Körper sind dabei gleich gerichtet, so dass immer nur ein Thier mit Samen versehen wird. Eine Ausstülpung des sehr kurzen Penis habe ich nicht beobachtet. Bei der Begattung geht viel Samen verloren. Moquin-Tandon, gestützt auf eine Angabe von Bojanus, nimmt an, dass die Körper der *Hirudineen* bei der Begattung entgegengesetzt gerichtet sind. Indess hat schon Ébrard*) in seinem sehr lesenswerthen Werke die Begattung von *Hirudo medicinalis* mit gleicher Richtung der Körper vor sich gehen sehen. Auch Iijima hat dies bei *Nephelis* bestätigt. Man kann die Begattung leicht beobachten. Wenn man zwei Thiere, welche 3-4 Tage isolirt waren, vereinigt, findet die Begattung sofort statt. Bei der von Iijima beobachteten Species findet ausser dieser Begattung eine andre Art statt, indem Spermatophoren von dem einen Thiere auf die Haut des andern befestigt werden. Bei unsern *Nephelis* habe ich diese Spermatophoren nie gesehen, obgleich mir das Vorkommen dieser Körper bei *Pontobdella* und *Piscicola* wohl bekannt ist."

6. *Piscicola*.

J. LEO. Verhältnisse der *Piscicola geometra*. Müller's *Arch. f. Anat.*, etc. 1835. p. 425.

“Die Begattung dieser Thiere geschieht auf folgende Weise. Die Fusscheiben zweier Individuen sind in einiger Entfernung von einander auf einer Ebene angeheftet und die Körper erhalten sich schwebend an den äusseren Öffnungen der Geschlechtstheile dergestalt Bauch an Bauch mit einander verschlungen, dass sie die Form eines X bilden, wobei aber das Kopfende jedes Thieres nach derselben Seite zurückgebogen ist, an welcher seine Fusscheibe haftet. Hinter der Umschlingung sind beide Körper bedeutend angeschwollen und dicht vor dieser Anschwellung sieht man in der Nähe der weiblichen Geschlechtsöffnung *eine weisse Masse hervortreten, die sich nach und nach vermehrt, und unter dem Microscope sich als ein Säckchen mit einer weissen, feinkörnigen und schleimigen Substanz erfüllt darstellt*. Ich glaube, dass *diese Masse ohnerachtet des häutigen Ueberzuges dennoch nichts anders als der aus den weiblichen Geschlechtstheilen überfliessende männliche Same ist, dessen Oberfläche aber wahrscheinlich durch den Einfluss des Mediums zu einer Haut gerinnt*. Dass die Ruthe in die weibliche Geschlechtsöffnung eindringt bemerkt man erst, wenn sich die Thiere von einander durch bewirkte Störung trennen, in welchem Falle dieselbe dann eine Zeit lang steif hervorsteht, wie es abgebildet ist.”

Leo's description of the spermatophore falls considerably short of being a discovery, and his remarks about the penis are wide of the mark. He seems to have mistaken the proboscis for a penis, as Leydig has pointed out.

T. BRIGHTWELL. Ueber die *Hirudo geometra*, Linn., und einige andere Arten von Susswasser-Egeln. Froiep's *Neue Notizen*. XXII, No. 467. 1842. p. 65.

Somewhat later than Leo, Brightwell again saw the spermatophore of *Piscicola* (“weisse Substanz”), but without understanding it. During copulation, as Brightwell puts it,

“Man bemerkte auf jeder Seite des Theils, wo die Körper ihre Vereinigung bewirkten, *eine weisse Substanz*. So blieben die Thiere gewöhnlich mehrere Stunden, in einem Falle sogar den ganzen Tag ueber verbunden. Als sie sich von einander trennten, löste sich von den Stellen, mit denen sie aneinandergehangen hatten, *eine weisse, spinnewebenartige Substanz ab*, welche sich in einem Falle wie ein Ei ausnahm, sich aber bei fernern Beobachtungen als ein Theil des Häutchens herausstellte,

von welchem die Eier umhüllt sind. Innerhalb 24 Stunden nach dem Begattungsacte wurden Eier gelegt."

FRANZ LEYDIG. Zur Anatomie von *Piscicola geometrica* mit theilweiser Vergleichung anderer einheimischer Hirudineen. *Zeitschr. f. w. Zool.* I. 1849. p. 124.

"Bei der Begattung stülpt sich aus der männlichen Geschlechtsöffnung eine Blase, welche die Ausmündungsstelle der Ductus def., sowie einen Theil der gelappten Drüse enthält. Dieser hervorgestülpte Theil gibt an die weibliche Geschlechtsöffnung die Samenmasse ab, welche als weisslicher Körper auch nach der Begattung an der weiblichen Genitalmündung sitzen bleibt. Die weissliche Masse, näher untersucht, erweist sich als *eine gedoppelte Blase mit doppeltem Stiel*, an welcher die Membran und die Stiele als aus dem Secret der gelappten Drüse bestehend, erkannt werden. Im Innern sind die Spermatozoiden in schön gelockter Weise geschichtet enthalten, so dass man wohl den ganzen weissen Körper als Spermatophoren bezeichnen kann. Schon im Ductus def. brünstiger Individuen lagern sich die Spermatozoiden zu solchen gelockten Bündeln zusammen, wie man sie nacher in den Spermatophoren findet. Es braucht also nur das Secret der gelappten Drüsen die Spermatozoiden bei der Ejaculation zu umhüllen, um die treffenden weissen Körper zu bilden. *Aus den Spermatophoren, welche halbe Tage lang nach der Begattung an der weiblichen Geschlechtsöffnung sitzen bleiben, bewegen sich die Spermatozoiden in den Eierstocksschlauch und dringen bis zu dessen blindem Ende vor. Ein solcher Eierstock mit eingewanderten Spermatozoiden hat ein schon dem blossen Auge wahrnehmbar verändertes, weissliches Aussehen.*"

Schneider confirms Leydig's account, and adds *Pontobdella* to the list of leeches which attach their spermatophores to the exterior. The definite location of the spermatophores in *Piscicola* is noteworthy, in comparison with *Nephelis* and *Clepsine*. Leydig assumes that the spermatozoa pass through the female genital pore into the ovaries; but he gives no proof of this. Perhaps his persuasion that it must be so, was the only reason he had for concluding that it was so. Schneider expressly states that the spermatophores of *Pontobdella* are attached to any point of the exterior *except at the genital pore*.

ANTON SCHNEIDER. Das Ei u. seine Befruchtung. 1883. pp. 32, 65.

PISCICOLA GEOMETRICA. — "Zwei Exemplare heften sich mit dem hintern Saugnapf fest, in entgegengesetzter Richtung und in der Entfernung, dass sie sich mit dem Vorderende erreichen können. Sie

krümmen das Vorderende und haken sich in einander. Solange die Reife der Eier nicht eingetreten, ist dies nur ein Vorspiel der Begattung, denn man findet in den Eileitern keinen Samen. *Dagegen werden bei dieser Gelegenheit Spermatophoren abgesetzt, welche man häufig auf dem Boden der Gefäße findet.*"

7. *Pontobdella*.

SCHNEIDER, *l.c.*

"Die Spermatophoren von *Piscicola* und *Pontobdella* sind kurze keulenförmige Röhren, deren Wand aus agglutinirten¹ Spermatozoen besteht, während das Innere mit freien Spermatozoen erfüllt ist. *Leydig hat dieselben bei Piscicola entdeckt und auch nachgewiesen, dass sie bei der Begattung an die weibliche Geschlechtsöffnung befestigt werden.* Wie schon oben bemerkt, werden diese Spermatophoren schon gebildet und abgelegt zu einer Zeit, wo die Thiere noch nicht geschlechtsreif sind und kein Samen in die Eileiter eintritt. Man findet die Spermatophoren dann auf dem Boden der Gefäße, worin die Thiere leben.

"Aehnlich wird die Begattung bei *Pontobdella* vor sich gehen. *Zur Zeit als ich Pontobdella lebend beobachtete [April in Triest], fand keine Begattung, sondern nur das Absetzen von Spermatophoren statt. Die Thiere befestigten sich dieselben gegenseitig an beliebige Körperstellen, nur nicht an die Geschlechtsöffnung.*"

8. *Hirudo*.

The ten-eyed leeches (*Hirudo*, *Aulostoma*, etc.) all have a well-developed intromittent organ, and observers now agree that in copulation the male organ of one individual is inserted in the female orifice of the other, and that fertilization is one-sided, not reciprocal. No one, so far as I know, has ever reported external spermatophores, and there is no reason to suppose that hypodermic impregnation ever occurs in the Gnathobdellidæ. Some of the older authorities maintained that fecundation was reciprocal, and Moquin-Tandon, as late as 1846, declared this to be a settled fact. Ébrard, however, disputes this point, and brings many facts to show that only one individual is fertilized at a time. Perhaps the conflicting testimony warrants the suggestion that fecundation may sometimes be reciprocal, at other times not. If copulation happened between two individuals equally ready to fecundate reciprocally, there

¹ An error corrected by Vejdovsky.

would be no difficulty in their doing so, provided the position taken were such as to admit of it. On the other hand, if one individual only were ready to discharge the male function, only one individual would be fecundated. I incline to take this view, as it offers a complete reconciliation of otherwise contradictory observations.

Ébrard's experiments in isolating leeches are not only interesting *per se*, but also in connection with the results before given of isolation of Clepsine.

A. MOQUIN-TANDON. Monographie des Hirudinées. 1846. pp. 166-68.

"Bibiéna, Thomas, Vitet, Mérat, Derheims et Fée, ont pensé que les Hirudinées se reproduisaient sans accouplement réciproque. Suivant Filippi, les *Glossiphonies* seulement sont capables de se féconder toutes seules.

"Weser, Cuvier, Carena, Virey et Blainville, ont admis, d'après la structure des organes sexuels, que chaque individu était incapable de se reproduire sans s'accoupler avec un autre. Leur opinion a été trouvée conforme à la nature, après les observations de Hebb et de Evans de Worcester (cités par Johnson), qui ont fait connaître que l'acte du coït se passait, chez ces Annelides, de la même manière que dans les Arions et les Hélices. Depuis cette époque, Kuntzmann, Bojanus, Odier et plusieurs autres naturalistes, ont eu l'occasion de voir des Hirudinées au moment de l'accouplement, et ont confirmé les observations des deux savants anglais.

"Dans l'accouplement des *Sangsues* médicinales, deux individus se rapprochent, ventre contre ventre et en sens inverse, de telle sorte que la ventouse orale de chacun est tournée, ou à peu près tournée, vers la ventouse anale de l'autre. On conçoit que, dans cette position respective, les organes génitaux se trouvant également situés en sens inverse, de manière que chaque verge doit se rencontrer en face d'une vulve. Les deux individus s'enlacent, et l'accouplement a lieu (Bojanus). Quelquefois les *Sangsues* s'attachent ensemble par leurs ventouses anales et laissent pendre librement leur partie antérieure (Burdach). Kuntzmann a cru reconnaître que, dans l'union sexuelle, les deux verges sont entortillées en spirale comme celle des Hélices; cette disposition est sans doute accidentelle (Bojanus).

"Le docteur Gaspard a prétendu que, dans chaque accouplement, un seul individu fécondait l'autre, lequel, après vingt-cinq ou trente jours, fécondait le premier dans un autre accouplement. Il est bien démontré aujourd'hui, par l'observation, que le coït est réciproque comme celui des Escargots.

“Johnson a observé l'accouplement de la *Néphélis*, et a reconnu qu'il était entièrement semblable à celui de la *Sangsue médicinale*.”

ÉBRARD. Nouvelle Monographie des Sangsues Médicinales. Paris, 1857. pp. 104, 105.

“Comment se fait-il donc que Bibiéna, Thomas, Vitet, Mérat leur aient accordé la faculté de se reproduire sans accouplement préalable? *Ils ont probablement été trompés par cette circonstance qu'une sangsue produit des cocons féconds trois, six, huit, et même dix mois après avoir été tenue isolée.* C'est là un fait dont il ne m'est pas permis de douter, quoique l'intervalle séparant l'accouplement des sangsues de la pose ou production des cocons ait été évaluée par divers auteurs à trente ou quarante jours.

“J'ai déjà publié, en 1851, l'histoire d'une sangsue verte de Hongrie qui, renfermée seule vers le 15 mai 1850, produisit le 15 et le 27 août des cocons féconds.

“En 1852, soupçonnant que les cocons de sangsues ne donnant pas le jour à des filets proviennent (de même que cela arrive pour les œufs stériles des femelles d'oiseaux tenues en cage) de sangsues ne s'étant pas accouplées, et, voulant m'en assurer, je plaçai, le 15 septembre, sept sangsues vaches dans autant de bocaux contenant de la terre et de la mousse, une sangsue dans chaque bocal (je les gorgeai et je les changeai de terre en mars (1853). Six de ces sangsues posèrent des cocons aux mois de juillet et d'août suivant, c'est-à-dire *après neuf à dix mois d'isolement, et, à mon grand étonnement, des filets sortirent de ces cocons.*

“En face de ces résultats si inattendus, je me demandai, je l'avouerais franchement, si les sangsues, tout en s'accouplant, ne pouvaient pas produire des cocons sans un accouplement préalable, ou plutôt si un rapprochement ne suffisait pas pour plusieurs années. Je me livrai, en conséquence à de nouvelles expériences : —

“1° Je continuai à tenir séparés quatre des sangsues précédentes qui étaient isolées depuis un an, et deux autres sangsues qui l'étaient depuis deux mois, depuis le mois de juin, et avaient récemment posé des cocons ; *toutes furent stériles l'année suivante*, quoique j'eusse pris soin de les gorger légèrement aux premiers jours du printemps.

“2° Je réunis, le 17 septembre, trois des sangsues ayant été tenues isolées depuis un an, puis je les séparai le 20 octobre. Je séparai le 17 septembre quatre autres sangsues qui étaient renfermées depuis quelque temps dans le même bocal. Une de ces sangsues périt, cinq des autres posèrent des cocons féconds pendant les mois d'août et de juillet de l'année suivante.

“Il est dès lors évident, ce me semble, que *les sangsues ont besoin de*

s'accoupler chaque année pour être fécondées, mais que, semblables en cela à plusieurs insectes, elles peuvent ne se reproduire que huit ou neuf mois après avoir été fécondées. Ce fait de la reproduction ayant lieu dans une année autre que celle de l'accouplement a d'ailleurs son analogue chez une hirudinée non médicinale ; car les glossiphonies [Clepsine] que, aux premières chaleurs du printemps et peu de jours après leur sortie de leur retraite hivernale, portent souvent déjà des œufs sous l'abdomen, ont certainement été fécondées dans l'année précédente."

9. *Aulostoma*.

EBRARD, *l.c.* p. 67.

"Deux aulastomes que j'ai trouvées accouplées étaient rapprochées tête à tête. Une seule fécondait l'autre ; car je n'aperçus qu'une seule verge lorsque je les séparai. L'une d'elles posa son premier cocon, œuf polysperme, quinze jours avant l'autre annélide."

10. *Macrobdella*.

Copulation has not, so far as I know, been observed in our common *Macrobdella*. That copulation occurs there can be little doubt. These leeches are remarkable for having so-called "copulatory glands," opening on the ventral side, a few rings behind the ♀ pore. It was Leidy who suggested that these glands are "provided for the adherence of individuals in sexual intercourse," and their position supports this view. This peculiarity makes it all the more desirable to witness the process of copulation. From what we know of other leeches, it is probable that individuals captured in early spring, and kept some days in isolation, would, on being brought together, very soon copulate.

OBSERVATIONS ON *CLEPSINE PLANA* (*n. sp.*).

The specimens on which my observations were first made were two large species obtained from Charles River, at Watertown, near Cambridge, in September, 1884. I am unable to identify them with any species hitherto described. Five of the nine individuals captured were dark brown, variously marked with yellow above, and with twelve or thirteen longitudinal lines below ; the remaining four were yellowish brown both above and below.

Clepsine parasitica, as described by Say¹ and Verrill,² agrees in certain features closely with the dark species, and Verrill's *C. papillifera* var. *carinata* may be identical with the light species. The descriptions, however, do not point out any characters which can be relied upon for identification.

The dark specimens, to be described in the following paper under the name *C. plana*, were ready to copulate whenever they met; but they always avoided the light species, which may be provisionally designated as *C. carinata*. The latter showed no disposition to copulate until early the following spring. They remained quiet during the winter; but on being started up in May, they began to deposit sperm-cases.

The observations which follow are confined mainly to *C. plana*. Two of this species bore young still stuffed with yolk, and another had eggs in its ovaries that were nearly mature.

When I first placed these Clepsines in a dish together, I noticed several long white bodies attached by one end to the dorsal surface of one or two individuals. I pulled them off for examination, thinking that they were parasites of some kind. Putting them under the microscope, I saw, to my great surprise, a stream of spermatozoa slowly issuing from the end that had been detached. At first I could hardly believe that these sperm-cases belonged to the leech, never having detected the animal in the act of depositing them, and not suspecting that they could discharge their contents through the skin. My curiosity having been thus aroused, I watched the leeches more closely, and soon had an opportunity to see the whole operation. The leeches were moving about as they usually do when first captured, before becoming wonted to new quarters. One individual, coming in contact with another, fixed itself by its oral sucker to some convenient point, and then, while pressing its protruded male pore against the back of its fellow, planted a fresh sperm-case. During the operation, which lasted only a few seconds, the body in the region of the genital pores was more or less constricted, somewhat as it is in the act of forming an egg-cocoon. The constriction seemed to be the expression

¹ Thomas Say: *Major Long's Expedition to the Source of St. Peter's River, etc., in 1823*. Vol. II, Appendix, p. 14. Keating's Compilation, London, 1825.

² A. E. Verrill: *Synopsis of North-American Fresh-water Leeches*. Professor Baird's Report for 1872-73.

of an effort to press the sperm-case firmly to the surface of attachment, and very likely the case was filled with spermatozoa by the same act. After a few moments of steady pressure, — just long enough to allow the sticky secretion to “set,” — the leech released its head and slowly drew back, allowing the spermatophore to be gradually *pulled* out of the two sac-like ends of the *vasa deferentia*. I saw this operation repeated several times by the same individual at intervals of about thirty minutes.

Among twenty or thirty spermatophores, I found only one on the ventral surface, and this was near the margin of the body; the rest were attached to the dorsal side, sometimes between two rings, sometimes in the middle of a ring, without any discrimination of place, so far as I could see.

Although the sperm-case is formed in two distinct sacs, uniting in a common pore, its two halves are firmly glued together, as the result of being pulled out through the single pore, while they are still in an adhesive condition. The moment they are set free, they are hardened by the action of the water, and only the small free ends sometimes remain distinct and separate.

One of the spermatophores first deposited measured 8 mm. in length and 1 mm. in width. Some of the last obtained measured only 3 mm. or even less. Repetition of the act seemed to exhaust the individual's power of forming spermatophores. Widely as they varied in size, they always showed essentially the same form as that shown in Fig. 4, *a* and *b*.

In the spermatophore we may distinguish (1) a short, constricted, basal portion with a single tubular lumen, formed in the median unpaired portion of the male organs; (2) an elongated body with a double saccular lumen, formed in the enlarged end-portions of the *vasa deferentia communia*; and (3) a free end, consisting of two distinct parts, adherent or separate, with lumen closed, or reduced to a narrow line, formed in the ends of the ejaculatory ducts (*d*) at the point marked *w* in Fig. 5. The wall of the spermatophore, which is thickest at the base and thinnest in the saccular body, is composed of two well-defined layers: an outer, thin, transparent, finely striated, non-stainable, cuticular-like layer (Fig. 2, *o*), which appears to fill the angles between the two halves of the case (Fig. 4 *b*), and to serve as a medium whereby the case is firmly glued to the

surface ; and an inner, denser, thicker, stainable layer (Fig. 2, *i*). The outer layer is so extremely thin over the saccular portion of the fresh spermatophore that it is difficult to recognize it ;¹ but it is easily demonstrated on sections. In the basal portion, this layer thickens, and then expands to form a broad base, so closely applied to the underlying cuticula as to form almost a continuum with it (Fig. 2). The striations of this layer may be due to the pull given to the sac as it is liberated from the genital pore, or more probably, as I think, to its mode of formation by numerous gland-cells.

When first placed, the spermatophore usually stands nearly perpendicular to the surface. It is tough and elastic, and considerable force is required to detach it. The skin of the leech around the place of attachment is at first strongly corrugated, as if by contraction ; but this appearance gradually passes away after the sac is emptied, although the sacs often remain for several days, or even weeks. Whether they are ultimately dissolved, or shed with the cuticula, or drop off as the result either of vital processes in the underlying skin, or of the solvent action of water, I am unable to say.

The mouth of the fresh spermatophore is completely plugged with a peculiar secretion (Fig. 4, *a*, *gs*), made up of elongated elliptical or spherical corpuscles (Fig. 4, *c*), varying from 0.02 mm. in diameter to much smaller dimensions. These bodies dissolve in water in the course of a few minutes. At first appearance, they are coarsely granular, but rapidly become perfectly homogeneous and transparent, and, growing paler and paler, fade away by insensible degrees. At first I took these bodies to be cells, as some of them appeared to be nucleated ; but having traced them to their origin in glands of a definite region of the *vasa deferentia*, I now think that the nucleus-like centre (Fig. 4, *c*) merely marks the depth to which the water had penetrated at the moment of examination. Their great variation in size is also in harmony with their origin as globular secretions.

This granular secretion probably serves a double purpose : first, to protect the spermatozoa inclosed in the saccular portion against contact with water ; and secondly, as a means of opening and clearing the way for the safer penetration of the sper-

¹ Exposed to acetic acid, it swells, and is thus made evident without the aid of sections.

matozoa. This mass is expelled through the skin in advance of the spermatic elements; and the disappearance of the pigment and the clarification of the tissues at the point of penetration, all of which is noticeable in sections (Fig. 2), suggest that it may have a softening effect on the tissues.¹ This, however, is pure conjecture. With plenty of material, the action of this secretion on fresh pigmented tissue might possibly be determined experimentally; but thus far I have not tried this.

If the leech is placed under a magnifying power of twenty or forty diameters, immediately after receiving one of the spermatophores, one may see the spermatozoa slowly flowing from the narrow mouth of the case through the skin. In the course of an hour the greater part of the contents has escaped, and the case itself is reduced to less than half of its original diameter. As soon as the case is planted, it begins to shrink; and this contraction, induced by the action of the water, is probably what forces the spermatic fluid through the skin. When the sac is first placed, the spermatozoa may be seen through the wall united in close bundles. Soon after deposit, as one may see towards the free end of the sac, these bundles begin to swell up, and the individual spermatozoa begin to show themselves. The appearance might raise a suspicion that a part of the spermatozoa undergo histolytic changes, serving by expansion as a means of expelling the rest, somewhat as described by Gruber in the Copepoda (v. extract). I think, however, that Leuckart's suggestion in regard to the spermatophore of *Astacus* is the explanation to be adopted here, as it is perfectly certain that the sperm-case gradually contracts as its contents escape. I find that a few spermatozoa are always left in the case after it has reached the limit of contraction, showing that the expelling force ceases to act after this. As distinct spermatozoa were found in a sperm-sac two days old, I infer that the sac is water-proof. If a fresh sac be detached and exposed to the pressure of a cover-slip, the sperm is rapidly driven out in the form of a white flaky string, consisting of a viscid fluid, with numerous bundles of spermatozoa.

In order to learn precisely where the spermatophore is formed, as well as the origin and relative positions of the various elements with which it is to be charged, it will be necessary to

¹ Isjima's observations on *Nephelis* favor this view.

examine briefly the form, structure, and contents of the male efferent ducts. A glance at Fig. 5 will show that these ducts are differentiated into a number of different regions, each of which seems to have a special function. Beginning with the pore, which lies between the tenth and the eleventh ganglia, we find a very short median tube, which bifurcates beneath the ventral cord, giving rise to two diverging horns (*s*), which are continued into a convoluted tube (*w*, *g*, *d*) of nearly uniform, but much smaller, diameter; then follows an enlarged sigmoid coil (*vs*), and finally the long narrow tube (*vd*), which receives the six short testicular ducts (*vd*). The sigmoid portion is a thin-walled reservoir completely filled with sperm-bundles, fulfilling the



FIG. 1.—Section from the posterior half of the *ductus ejaculatorius*, showing bundles of spermatozoa (*sp*) massed together, and a few granular corpuscles (*gc*), probably secretions from the anterior, glandular half of the duct. The muscular layer (*m*) is strongly developed, and the large lumen is lined with a thin epithelium (*ep*).

office of a *vesicula seminalis*. The convoluted portion connecting the *vesicula seminalis* with the terminal horn-like enlargement (*s*) appears externally to be a nearly uniform tube, and is usually called the *ductus ejaculatorius* (*d*). But an examination of the structure and contents of this portion reveals the fact that it is really differentiated into two parts which fulfil different functions. The posterior half (*d*) has a thicker muscular wall, and a much larger lumen than the anterior half (*w* and *g*); it is lined with a thin epithelium, and is filled with spermatozoa. In the anterior half this epithelial lining takes the form of long columnar gland-cells, radially disposed, with the

nucleated ends next to the muscular wall. The reduction of the lumen in this part is due to the development of this glandular epithelium. It is in this glandular portion that the granular corpuscles (*gc*) which plug the spermatophore are produced.

I have a series of sections of this region, showing the gland-cells fixed in the very act of secreting these corpuscles. The corpuscles are somewhat pyriform in shape, with the smaller end tapering to a fine thread, which is connected with the central end of the producing cell (Fig. 2, *gc*). As soon as the corpuscle is fully liberated, it assumes a more or less elliptical form. These corpuscles sometimes nearly fill the whole lumen of the duct; sometimes they lie in masses that resemble clusters of

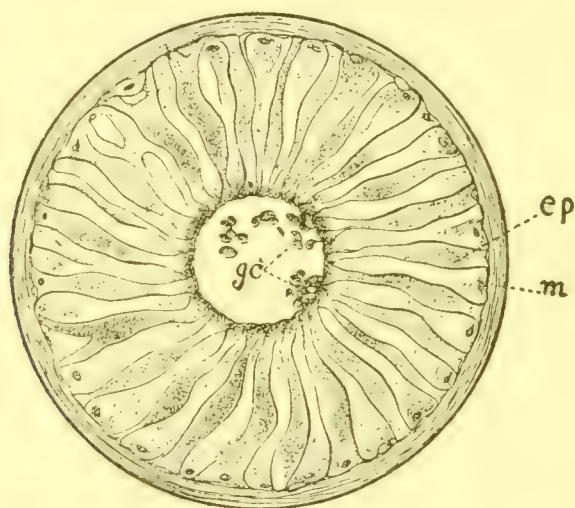


FIG. 2. — Section from the glandular anterior half of the duct, in which the lining epithelium (*ep*) is transformed into radial, pyramidal gland-cells, with nuclei at the external bases. Granular corpuscles (*gc*) in process of formation. Muscular layer thinner than in Fig. 1. The lumen of the canal is very much reduced by the thickening of the epithelial lining.

blood-corpuscles. I find a few such clusters in the posterior non-glandular region (*d*), but I doubt if they move backward to this part as a regular thing. They were more probably thrown backward from the place of origin by irregular, peristaltic contractions during the process of killing. At the anterior end (*w*) of the corpuscle-secreting region, the glandular lining becomes thicker, and passes imperceptibly into the still thicker lining of the terminal horns. I find no corpuscles at this level (*w*), and it is here that the free end of the spermatophore is evidently secreted. The saccular part of the spermatophore, as before noticed, is secreted in the horns (*s*), while the narrower base is

formed in the median unpaired tube lying beneath the nerve-cord.

The foregoing facts enable us to form some idea of what probably goes on each time a spermatophore is produced. My conjecture is as follows: As soon as the sperm-case is ready for the reception of its burden, the corpuscular secretion and the spermatic fluid contained in the convoluted tube (*w*, *g*, and *d*) are driven forward into it. The contents of this whole tube between the horn (*s*) and the vesicle (*vs*) is probably required

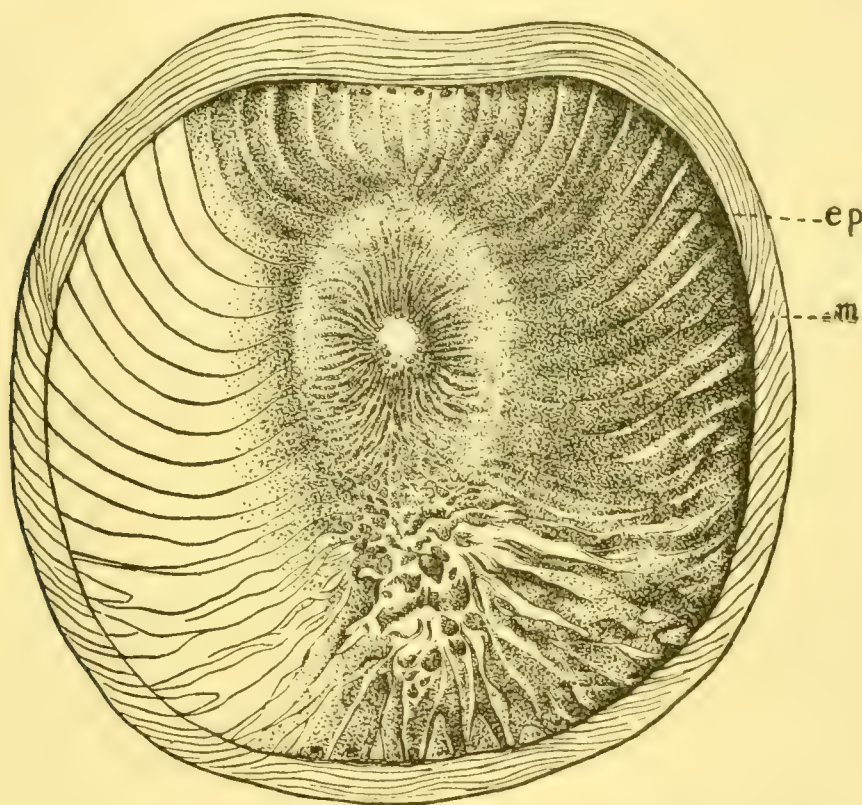


FIG. 3. — Section of the terminal horn of the efferent duct, just below the point of union with the ejaculatory coil. The epithelial layer is still more strongly developed. The cells are fixed in the act of secreting a spermatophore; the secretion still shows its origin from numerous single cells.

for a single charge. The spermatic fluid would sweep the corpuscular mass before it, and leave it in the basal portion of the sperm-case. Whether any portion of the contents of the *vesicula seminalis* is needed in addition to that of the *ductus ejaculatorius* to complete a single charge, is a matter of doubt. I am inclined to think that the charge is measured off each time in the ejaculatory ducts, and that the contents of the *vesiculæ seminales* is brought forward only to replace what has been ejected.

In this connection I desire to call attention to a peculiar feature of the ovaries of this species. A little behind the point of union of the two ovaries (Fig. 5) is seen a large cæcal diverticulum (*ca*) directed obliquely forward and outward, and terminated by a fibrous prolongation. I have not examined the minute structure of this part. I wish here merely to suggest that the ovarian sac of *Clepsine* may have been derived from a looped form like that of *Nephelis*. The free end of the loop in *Nephelis* (according to Iijima, *l.c.*) terminates with a fibrous prolongation similar to that of the cul-de-sac of *C. plana*. A drawing of the same organs in *C. marginata* (Leipzig), made when I was in Leuckart's laboratory, shows this same fibrous prolongation, arising, not from a cæcal appendage, but from the anterior angle of the simple ovary.

NOTES AND EXTRACTS RELATING TO THE PHENOMENA OF
IMPREGNATION BY MEANS OF SPERMATOPHORES IN THE
TURBELLARIA, THE ROTATORIA, DINOPHILUS, THE CHÆTO-
PODS, PERIPATUS, ETC.¹

I. *The Turbellaria.*

ARNOLD LANG. Der Bau von *Gunda segmentata*, etc. *Mitt. a. d. Zool. St. z. Neapel.* III, 1 and 2. pp. 222-24. 1882.

“Die Endapparate der Geschlechtsorgane sind bei den Polycladen äusserst mannigfaltig gebaut. Betrachten wir diese Thiere als kriechende Coelenteraten, so darf uns diese Mannigfaltigkeit nicht wundern, denn *dann sind die Polycladen die ersten Thiere, bei denen eine wahre Copulation vorkommt.* Dann setzt uns auch ein gewisser Copulationsmodus, den ich bei mehreren Arten verschiedener Gattungen von Polycladen beobachtet habe, nicht mehr in zu grosses Erstaunen.

“Lange Zeit hatte ich nämlich vergeblich nach einer Erklärung der eigenthümlichen Thatsache gesucht, dass bei *Thysanozoon* 2 männliche Öffnungen und 2 Penes vorkommen, daneben aber eine einzige weibliche Öffnung existirt. Für die Erklärung war wenig gewonnen, als ich bei andern Gattungen von Polycladen dieselbe Thatsache entdeckte und sogar bei einer neuen Art und Gattung bei einem jungen *Thiere* 9, bei

¹ As the literature on the subject of hypodermic impregnation has not hitherto been collected, I have given at length such reports as seemed to me to be of especial interest. Doubtless I may have overlooked much that would be of value in this connection. What I have given will suffice to show that the subject is worthy of further investigation.

einem 15 *Penes* in zwei seitlichen Längsreihen angeordnet vorfand. Daneben bestand immer nur eine einzige weibliche Öffnung. Auf die richtige Spur brachte mich endlich eine Beobachtung, die ich vor zwei Jahren an einer in die Nähe der Gattung *Leptoplana* gehörenden Art wiederholt anzustellen Gelegenheit hatte. *Die meisten Exemplare dieser Art, die mir von den Fischern gebracht wurden, trugen nämlich eine geringere oder grössere Anzahl weisser, ziemlich resistenter Fäden, die ohne irgend welche Regelmässigkeit auf der Bauch- oder auf der Rückseite, oder auf beiden zugleich im Körper der Thiere befestigt waren. Zuerst dachte ich, dass ich es hier mit Parasiten zu thun habe. Die genaue Untersuchung ergab jedoch, dass die erwähnten Anhänge weiter nichts sind, als Spermatophoren.* Sie bestehen aus einer structurlosen, resistenten Hüllmembran, welche in ihrem Innern einen Haufen Spermatozoen enthält. An Schnitten und an lebenden Thieren zeigte es sich, *dass diese Spermatophoren mit Gewalt und unter Zerreißen des Epithels der Basalmembran und der Musculatur in den Körper der Thiere eingesteckt worden waren. Der Samen ergiesst sich von den Spermatophoren in alle Hohlräume des Körpers, so dass man öfter im Lumen der Darmäste Sperma in reichlicher Quantität antrifft. Zufällig gelangt er auch in die Eileiter, die im ganzen Körper sich verästeln. Hier findet die Befruchtung der Eier statt.*

“Die Untersuchung des Penis ergab Resultate, die mit dieser eigenthümlichen Art der Copulation vollständig in Einklang stehen. Er besteht aus einem hintern drüsigen Theil, welcher offenbar die structurlose Hülle der Spermatophoren bildet und aus einem vordern sehr musculösen und langgezogenen Theil, dessen Höhlung spiralig gewunden ist. In Folge dieser Beschaffenheit des Penis kann das Spermataphor wie ein Korkzieher in den Körper des die Misshandlung erleidenden Individuums eingebohrt werden. *Die weibliche Öffnung dient bei dieser Species ausschliesslich zur Eiablage. . . .*

“Diese eigenthümliche Copulationsart, neben der bei vielen Arten *auch eine richtige Begattung vorkommt*, erklärt nach meiner Ansicht so vollständig das Vorkommen mehrerer *Penes*¹ neben einer einzigen weiblichen Geschlechtsöffnung, dass ich weitere Commentare für unnöthig halte. *In wie weit sie als eine ursprüngliche zu bezeichnen ist, wage ich nicht zu entscheiden. Jedenfalls scheint es mir charakteristisch, dass sie ausschliesslich bei denjenigen Thieren vorkommt, die ich für die ältesten Bilaterien halten muss und bei denen wahrscheinlich zum ersten Male die Befruchtung durch eine Copulation eingeleitet wurde.*”

¹ In *Histriodrilus Benedeni*, Foet. (*Histriobdella homari*, P. J. v. Ben.), Alexandre Foettinger finds three penes. It is possible that this “archiannelid” accomplishes the act of fertilization in the same manner as the pluri-penial Polyclads. Arch. de Biol., Vol. III, pp. 482–83 and 510. 1884.

ARNOLD LANG. Die Polycladen. *Fauna und Flora des Golfes von Neapel*. XI. Monographie. 2d Hälfte. pp. 636–38. 1884.

“Ich habe nur bei einer einzigen Art, nämlich bei *Stylochus neapolitanus*, den Vorgang der Copulation im gewöhnlichen Sinne des Wortes, d. h. die Einführung von Sperma in den weiblichen Begattungsapparat durch den männlichen beobachtet. . . .

“Es ist sehr wahrscheinlich, dass noch bei vielen anderen Polycladen sich eine normale Begattung vollzieht. Bei einer Reihe von Formen aber geschieht die Copulation in einer bis jetzt im Thierreiche ganz allein dastehenden, höchst merkwürdigen Art und Weise. Der Umstand, dass *Thysanozoon Brocchii* zwei Penes und zwei getrennte männliche Geschlechtsapparat besitzt, hatte meine Neugierde, zu sehen, wie sich bei diesen Organisations-verhältnissen die Begattung bei dieser Art vollziehe, schon lange wach gerufen. Diese wurde noch gesteigert, als ich noch andere Pseudoceriden mit doppeltem männlichen Begattungsapparat entdeckte, und gar erst, als ich den merkwürdigen *Anonymus virilis* mit seinen zahlreichen Penes, aber nur einer weiblichen Oeffnung auffand. Die genauere Untersuchung der Einrichtung und des Baues der Begattungsapparate dieser Formen brachte keine Aufklärung darüber, in welcher Weise bei ihnen die Copulation sich vollziehen könne, sie zeigte vielmehr, dass die ganze Organisation und Anordnung der in Frage stehenden Apparate für eine richtige Begattung so unpassend wie möglich ist. Den ersten Schritt auf dem Wege zur Aufklärung der Verhältnisse machte ich eines Tages, als man mir den prächtigen *Pseudoceros superbus* brachte. Ich setzte ihn in ein Bassin, in welchem sich mehrere schöne Exemplare, von *Yungia aurantiaca* und *Thysanozoon Brocchii* befanden. Das Thier kroch an den Wänden des Gefässes umher, stiess zufällig auf eine *Yungia*, wurde nun plötzlich sehr aufgeregt, liess seine beiden Penes weit hervortreten, und glitt über das Exemplar von *Yungia* hinweg. Bei seinem eiligen Umherkriechen traf es noch öfter mit Exemplaren von *Yungia* und *Thysanozoon* zusammen. Jedesmal wenn dies geschah, wurden die Penes hervorgestreckt, so dass ich mich veranlasst fühlte, die Individuen, über die der *Pseudoceros superbus* hinweg gekrochen war, aus dem Bassin heraus zu nehmen und zu examiniren. Da stellte sich heraus, dass alle diese Exemplare mehr oder weniger zahlreiche Wunden hatten, und zwar an allen möglichen Körperstellen, und in den Wunden fanden sich ansehnliche weisse Klumpen von Sperma (ich will hier noch beiläufig bemerken, dass sowohl bei *Pseudoceros superbus* als bei den anderen *Pseudoceriden* die Penes beim Schwimmen häufig weit hervor gestreckt werden). Diese Beobachtung brachte mich zuerst auf den Gedanken, dass die männlichen Begattungsapparate der Polycladen neben ihrer eigentlichen Func-

tion auch noch die von Waffen zum Angriff oder zur Vertheidigung haben könnten; sie rief mir zugleich eine alte Beobachtung die ich gemacht hatte, in das Gedächtniss zurück. Ich hatte nämlich schon mehrere Male in den Aquarien, in denen ich *Thysanozoon* hielt, diese Thiere mit vorgestrecktem Penis aufgeregt herum und übereinander hinweg kriechen sehen. Es bot sich mir bald die Gelegenheit, diese Beobachtung wieder zu erneuern, und ich unterliess es diesmal nicht, nach dem Ereignisse die Thiere zu untersuchen. *Mein Erstaunen war gross, als ich fand, dass sich auch die Exemplare von Thysanozoon gegenseitig verletzt und Häufchen von Sperma in die Wunden abgelegt hatten. Dies brachte mich zum ersten Mal auf den Gedanken, dass wenigstens bei den Polycladen mit doppeltem oder vielfachem männlichen Begattungsapparat und einfacher weiblicher Geschlechtsöffnung die Begattung sich so vollziehe, dass die Begattungsglieder eines Individuums an irgend einer Körperstelle anstecken, Sperma in die Wunde entleeren, und dass dann das Sperma zufällig in die im Körper reich verzweigten Eileiter gelange.* Ich fand sodann auf Schnitten in der That bei vielen Pseudoceriden Sperma nicht nur in den Eileitern, sondern auch in *Darmästen, im Parenchym, etc.* Diese Art der Copulation erschien mir aber doch so eigenthümlich, so ganz verschieden von allem, was bis dahin bekannt war, und hauptsächlich so unnatürlich, dass sich immer wieder Zweifel an der Richtigkeit meiner Auffassung der oben beschriebenen Vorgänge in mir regten. *Diese Zweifel verschwanden aber vollständig, als ich die Entdeckung machte, dass bei Cryptocelis alba im männlichen Begattungsapparat Spermatophoren erzeugt werden, die dazu bestimmt sind, mit Gewalt in die Leibeswand anderer Individuen derselben Art eingepflanzt zu werden. Als ich zuerst Individuen von Cryptocelis alba bekam, die an den verschiedensten Körperstellen mit einer wechselnden Anzahl dieser weissen, fadenförmigen, zähen Spermatophoren besetzt waren, glaubte ich erst, dass es Parasiten seien, bis ich ein solches Gebilde öffnete und eine Unmasse von Spermatozoen von der Form derjenigen von Cryptocelis alba heraustreten sah.* Die Spermatophoren sind unter Durchbrechung des Epithels, der Basalmembran und der Musculatur so fest in den Körper eingepflanzt, dass sie sich bis auf ihre doppelte und dreifache Länge zu langen, dünnen Fäden ausziehen lassen, bevor sie sich loslösen. *Die durch das Einpflanzen der Spermatophoren hervorgerufenen Wunden lassen deutliche Narben zurück.* Man trifft sie bei zahlreichen Individuen an. Der grosse kräftige, äusserst musculöse Begattungsapparat von *Cryptocelis* erscheint seiner Function sehr gut angepasst.

“Spermatophoren werden auch noch bei anderen Polycladen producirt. Im Körperparenchym von *Prostheceraeus albocinctus* fand ich unzählige Häufchen von Samenfäden, deren Kopfen alle in einer

Ebene lagen und deren Schwänze nach einer und derselben Seite gerichtet waren. Ballen von Sperma fand ich auch sehr häufig vor dem Eingang zum weiblichen Begattungsapparat von *Leptoplana tremellaris*. Diese Spermatophoren unterscheiden sich von denen der *Cryptocelis alba* dadurch, dass sie nicht in eine Membran oder Kapsel eingeschlossen sind."

Id. ibid. 1st Hälfte. pp. 231-32.

"Wie im biologischen Theile auseinander gesetzt werden wird, dienen die männlichen Copulationsorgane mehreren Polycladen nicht zu einer eigentlichen Copulation im gewöhnlichen Sinne des Wortes, d. h. sie werden nicht in die weiblichen Copulationsorgane eingeführt. Sie dienen vielmehr diesen Formen dazu, den Körper eines anderen Individuums derselben Art an irgend einer Stelle anzustechen und den Samen in die so erzeugte Wunde zu ergiessen, oder eigens bereitete Spermatophoren mit Gewalt in den Körper des die Misshandlung erleidenden Individuums einzupflanzen. *Diese eigenthümliche, bis jetzt, so viel ich weiss, bei allen Thieren ganz allein dastehende Art der Begattung macht es verständlich, dass bei Pseudoceriden und Anonymiden neben einem einzigen weiblichen Begattungsapparat zwei oder mehrere männliche vorkommen.*

"*Ich erblicke in ihr aber ferner auch einen Vorgang, der auf die phylogenetische Bedeutung der Copulationsorgane der Polycladen vielleicht einiges Licht wirft.* Wenn, wie ich anzunehmen geneigt bin, die Polycladen aus Coelenteraten durch Anpassung an die kriechende Lebensweise hervorgegangen sind, so haben ihre Vorfahren keine Begattungsapparate besessen. Es bleibt also die Schwierigkeit, die Entstehung dieser oft so complicirten Organe bei den Polycladen zu erklären. Ich weiss sehr wohl, dass diese Schwierigkeit gegenwärtig noch nicht zu beseitigen ist, doch dürften vielleicht die folgenden Bemerkungen einen Fingerzeig abgeben, in welcher Richtung die Lösung der Frage zu suchen sein wird. *Die männlichen Begattungsapparate vieler Polycladen, ganz besonders diejenigen, welche an ihren Ende ein hartes Stilett tragen, stehen nämlich sicher nicht ausschliesslich im Dienste geschlechtlicher Functionen, sondern sie dienen auch als Waffen zum Angriff und vielleicht auch zur Vertheidigung.* Schon O. Schmidt, Hallez und v. Graff haben für die harten Stilette der männlichen Geschlechtsapparate gewisser Rhabdocoeliden diese Auffassung ausgesprochen, die ersten beiden haben dieselbe sogar durch directe Beobachtung erhärtet. Ich habe bei Pseudoceriden ebenfalls direct beobachtet, dass die Penes als Waffen gebraucht werden. Ein grosses und schönes Exemplar von *Pseudoceros superbus* sah ich in einem meiner Aquarien, in denen sich

mehrere Exemplare von *Thysanozoon Diesingii* und *Yungia aurantiaca* befanden, in grosser Aufregung umher kriechen und von Zeit zu Zeit die beiden Penes weit vorstrecken. Es kroch öfter über die anderen erwähnten Polycladen hinweg und brachte ihnen durch Vorstossen der Penes zahlreiche Wunden bei, in denen ich stets ein Häufchen Sperma vorfand. Ganz ähnliches habe ich zu wiederholten Malen auch bei *Thysanozoon Diesingii* beobachtet. Aber noch ein anderer Umstand spricht zu Gunsten der gelegentlichen Verwendung der Copulationsorgane als Waffen. Das Lagerungsverhältniss des männlichen Begattungsapparates von *Stylostomum* zu Pharyngealtasche und Pharynx bringt es, wie ich weiter unten nachweisen werde, mit sich, dass der Pharynx nicht vorgestreckt werden kann, ohne dass nicht auch der mit einem harten Stilett versehene Penis vorgestossen wird. Wenn wir uns nun ferner daran erinnern, dass bei der Begattung mehrerer Polycladen-Arten eine gewaltsame Verwundung der Individuen durch den Penis an den verschiedensten Körperstellen erfolgt, so liegt der Gedanke doch gewiss nahe, dass die Copulationsorgane der Polycladen ursprünglich Angriffs- und Vertheidigungswaffen waren, die erst secundär in den Dienst geschlechtlicher Functionen traten. Von diesem Gesichtspunkte aus ist das Vorhandensein einer grossen Anzahl von Begattungsorganen bei dem ursprünglichen Genus *Anonymus* sehr leicht erklärlich, und die oben erwähnte Begattungsweise erscheint uns viel weniger seltsam.

2. *Rotatoria*.

LUDWIG PLATE. Beiträge zur Naturgeschichte der Rotatorien. *Jen. Zeit. für Nat.* XIX. 1885. pp. 110–11.

“Dass die Spermatozoen bei den begatteten Weibchen frei in der perienterischen Flüssigkeit sich umhertummeln, ist eine von vielen Forschern wiederholt gemachte Beobachtung: aber wie sie hinein gelangen, ist von denselben nicht erkannt worden. Cohn und Brightwell konnten, da sie nur mit Lupen arbeiteten, weiter nichts bemerken, als dass die Männchen sich dicht an die Weibchen anhefteten, und ersterer vermutete bei *Hydatina* und *Conochilus* einen besonderen, in der Halsgegend befindlichen Genitalporus. Eyferth berichtet: ‘bei *Diglena catellina* habe ich die Anheftung (der Männchen) an die Kloakenmündung gesehen.’ Hudson¹ dagegen fand bei *Asplanchna Ebbesbornii* ‘ein Männchen, das mit der Spitze des Penis dem Weibchen anhing.’ Aber es war an der Aussenseite der Bauchflächenmitte und nicht an der Oviductöffnung.’ Alle diese widersprechenden Angaben erklären sich leicht aus den Beobachtungen, die im speciellen Teile

¹ Jour. Roy. Micr. Soc., Vol. III, Part 5, 1883, p. 622.

bei *Hydatina senta* geschildert wurden. Sie führten zu dem merkwürdigen und, soviel ich weiss, im ganzen Tierreich nur noch bei einigen Planarien vorkommenden Ergebnis, dass der Penis die Körperwandung des Weibchens bei der Copulation an irgend einer beliebigen Stelle durchbohrt, derselbe dagegen nicht, wie man erwarten sollte, in die Kloake gesteckt wird. Unter geeigneten Umständen vermag daher auch dasselbe Weibchen gleichzeitig von mehreren Männchen begattet zu werden. Da schon bei so vielen anderen Specien Sperma frei in der Leibeshöhle flottierend gefunden worden ist, kann kaum bezweifelt werden, dass auch bei diesen die Begattung in gleicher Weise vollzogen wird. Es fragt sich nun, ob wir annehmen dürfen, dass auch zu jener Zeit, als die Männchen wie die Weibchen mit Mundöffnung und Darm versehen und in ihrer ganzen Organisation noch nicht rückgebildet waren, der männliche Same auf dieselbe Weise in den weiblichen Körper gebracht wurde. Ehe bei *Seison*, dem einzigen Rädertier, dessen Männchen noch nicht retrometamorphosiert ist, die Copulation nicht beobachtet worden ist, lässt sich freilich die angeregte Frage nicht mit Sicherheit entscheiden. Da jedoch Claus von dieser Gattung glaubt mit Sicherheit behaupten zu können, dass die Samenfäden nicht frei in der Leibeshöhle, sondern in dem dünnhäutigen Ovar sich befinden, scheint es mir das Wahrscheinlichste zu sein, dass ursprünglich der Penis in die Kloake geschoben, und auf diesem allein natürlichen Wege das Sperma mit den Keimzellen zusammengebracht wurde. Wir müssen dann annehmen, dass mit der Rückbildung der Männchen oder vielleicht bewirkt durch dieselbe eine Änderung in der Art des Coitus eingetreten ist."

pp. 37-39.

"Um den Akt der Begattung zu beobachten, thut man gut, gleichzeitig eine grössere Anzahl (6-10) Männchen mit einem Weibchen in einem kleinen Tropfen zu isolieren. Die ersteren besitzen nämlich nicht die Fähigkeit, die Nähe des anderen Geschlechts zu wittern, auch nicht dann, wenn das siedende Gewimmel der Samenfäden, — welches manchmal erst am zweiten Tage nach dem Verlassen des Eis eintritt, — anzeigt, dass sich die Tierchen in einem begattungsfähigen Zustande befinden. Auch die Weibchen bekümmern sich nicht um die Männchen; beide werden lediglich durch den Zufall zusammengeführt, und bringt man daher 1 oder 2 Männchen mit einem oder wenigen Weibchen zusammen, so muss man oft Stunden lang warten, bis eine Begattung wirklich eintritt. Oft kommen beide Geschlechter vielfach mit einander in Berührung, ohne zu copulieren, wie auch schon Cohn mit der Lupe beobachtet hat, dass die Männchen 'die Weibchen umschwärmen, sich an diese anlegen, meist aber von diesen . . . wieder zurückgeschreckt werden.' Bei der Begattung wird merkwürdiger Weise der Penis des Männchens, nicht in

die Kloake oder in eine andere Öffnung der Cuticula geschoben, sondern durchbricht letztere an irgend einer beliebigen Stelle und befördert wahrscheinlich durch eine energische Contraction der Muskulatur des Hodens die stäbchenförmigen Körper und das Sperma in die Leibeshöhle. Während der Begattung Krümmt sich der Körper des Männchens so, dass die Bauchseite einen concaven Bogen darstellt. Die Zehen werden hierbei nicht gebraucht, sondern auch der Ventralfläche zugewendet, sodass, das ganze Tier eine halbmondförmige Gestalt annimmt und nur mit dem ausgestreckten Penis sich festheftet. Da eine besondere Genitalöffnung nicht vorhanden ist, kann ein Weibchen gleichzeitig von mehreren Männchen begattet werden, ein Vorgang, der in der Natur wegen der Seltenheit der letzteren wohl kaum vorkommen dürfte, den ich aber unter den oben angegebenen Bedingungen wiederholt beobachtet habe; so sah ich 2, 3, 5, und einmal eine noch grössere Zahl von Männchen (6–8) gleichzeitig mit demselben Weibchen copulieren. Es ist mir öfters aufgefallen, dass, wenn man Tiere beiderlei Geschlechts in einem kleinen Tropfen isoliert, dieselben zunächst längere Zeit gleichgültig an einander vorbeischwimmen; umschwärmt jedoch erst ein Männchen das Weibchen, so sammeln sich bald mehrere der ersteren um dasselbe, gleichsam als ob die Männchen sich gegenseitig bemerkten. Bei der Begattung wird der Penis nicht in seiner ganzen Länge durch die gebildete Öffnung der Cuticula in die Leibeshöhle hereingeschoben, sondern klebt nur äusserlich derselben an. Sie wird nur sehr klein sein, denn ich habe nie nach erfolgter Begattung Spuren derselben in der Haut wahrnehmen können. Leider kann ich nicht mit Sicherheit angeben, wie die Öffnung zum Übertritt des Sperma entsteht, da die stete Beweglichkeit der copulierenden Tiere die Untersuchung sehr erschwert. Aus der Beobachtung von Tieren, die gleichzeitig von mehreren Männchen begattet und in diesem Augenblicke durch ein Deckglas festgehalten wurden, glaube ich jedoch schliessen zu dürfen, dass die grossen Borsten an der Penisöffnung und die pfeilartigen Stäbchen, die wegen ihrer Lage im Vas deferens zuerst herausgepresst werden, die Körperwand des Weibchens durchbrechen. Alle diese zweifelhaften Punkte würden sich ohne Schwierigkeit an den grossen Asplanchnaarten lösen lassen. An demselben Genus würde man auch relativ leicht beobachten können, welchen Einfluss das Sperma auf die Bildung der Eier ausübt, ob die Begattung auch eine Befruchtung nach sich zieht oder ob, — wie ich glaube, — die Samenfäden in der Leibeshöhle der Weibchen sämtlich nach einiger Zeit zu Grunde gehen, und der Begattungsakt durch das Auftreten der Parthenogenese seine weiteren Folgen verloren hat. Es wäre dies ein in der Tierreihe nach unsern jetzigen Kenntnissen wohl einzig dastehender Fall, den man als einen 'rudimentaren Vorgang' in demselben Sinne bezeichnen könnte, wie man Organe, die ausser Function getreten sind, rudi-

mentär nennt. Leider wusste ich, als ich das Verhalten der Spermatozoen in der Leibeshöhle verfolgte, noch nicht, dass nur ein Teil der Geschlechtsorgane, nämlich der Eierstock, bei Entscheidung jener Frage in Betracht kommen kann und richtete meine Aufmerksamkeit daher nicht besonders auf den Vorderrand des Dotterstockes. Was man an den begatteten Tieren beobachtet, ist eigentlich nur sehr wenig. Das Sperma häuft sich zuweilen in einem Klumpen innen um die gebildete Hautöffnung herum an, flottiert jedoch in der Regel frei in der Leibeshöhle, verteilt sich zwischen allen Organen, *zwischen den Fäden des Gehirns so gut*, wie in der Nähe der Klebdrüsen und sammelt sich weder in der Umgegend der Geschlechtsorgane vorzugsweise an, noch habe ich je beobachten können, dass die Samenfäden den Versuch gemacht hatten, in dieselben einzudringen. Der Dotterstock eines gut genährten Tieres bildet nächst dem Darm das grösste Organ im Körper, und es ist natürlich, dass sich an seiner Oberfläche mehr Spermatozoen ansammeln als auf einem kleineren, z. B. der contractilen Blase. Übt die Fortpflanzungsorgane ferner eine besondere Anziehungskraft auf das Sperma aus (wie Cohn es bei *Conochilus* gesehen haben will), so müsste sich dasselbe vorn in der Nähe des Keimstockes ansammeln, was mir sicherlich nicht entgangen wäre, auch ohne zu wissen, warum gerade dieser Teil von den Samentierchen bevorzugt würde. Während die eben in die Leibeshöhle gelangten Spermatozoen sich zunächst noch sehr lebhaft bewegen und daher, wenn sie der Genitaldrüse dicht anliegen, durch das hin und her Schlängeln ihres Schwanzes leicht den Eindruck hervorrufen können, als ob sie sich in dieselbe einzubohren suchten, werden diese Bewegungen nach einigen Stunden immer matter. Dabei schwellen sie am vorderen Ende dick an, werden allmählich kugelförmig und im Innern vacuolisiert, kurz, *es ist offenbar, dass sie einen längeren Aufenthalt in der perienterischen Flüssigkeit nicht zu vetragen vermögen, sondern darin sterben und sich zersetzen.* Untersucht man ein begattetes Tier nach 24 Stunden, so nimmt man nichts mehr von denselben wahr.

“Die ausgesprochene Ansicht, dass sich die *Hydatina senta* ausschliesslich parthenogenetisch fortpflanzt, stützt sich vornehmlich auf den Mangel einer anziehenden Wirkung der Geschlechtsorgane auf das Sperma, und darauf, dass nie das Eindringen des Samens in jene beobachtet wurde.”

3. *Dinophilus*.

SIDNEY F. HARMER. Notes on the Anatomy of *Dinophilus*. *Studies from Morph. Lab. Univ. Cambridge*. Vol. I, pp. 49, 50. 1890. [Reprint from *Jour. Mar. Biol. Assoc. N. S.* Vol. I.]

“So far as I am aware, copulation has not hitherto been actually proved to take place in any species of *Dinophilus*. The proof that such a

process takes place in *D. tæniatus* is very readily obtained by merely placing a considerable number of individuals of both sexes in a small quantity of sea-water, as in a watch-glass. Under these circumstances, it is noticed, even a very short time after the animals have been placed together, that here and there a male is attached, by means of its penis, to the body of a female. In these cases, the terminal, conical portion of the penis is protruded through the generative pore, and *is passed into the skin of the female; spermatozoa are then seen to have passed, from the vesiculæ seminales, through the skin of the female, and to be accumulating themselves into a mass immediately beneath the perforation made by the penis.*

"There seems to be no localization of the spot at which spermatozoa can be introduced into the female. The penis can obviously be inserted into the skin at any point, as is shown by the fact that, in the cases actually observed, the point selected was sometimes in the region of the neck, in other cases far back in the body of the female, and in other cases near the middle of the body.

"The act of copulation has no relation to the maturity of the ova of the female, nor is it prevented by the fact that the female has already received an ample supply of spermatozoa by a preceding operation. It was extremely difficult to discover any female, in which ovaries were recognizably developed, which did not contain large numbers of spermatozoa in its body-cavity. These were observed in almost any part of the body of the animal, their position being probably partly dependent on the manner in which fertilization had been previously effected. The spermatozoa show, however, a great tendency to accumulate into a large, compact mass, situated in a space on the ventral side of the stomach. *In some cases it was observed that the female was receiving spermatozoa simultaneously from two males;* in others that while, for instance, fertilization was being effected near the posterior end of the body, a great mass of spermatozoa (obviously obtained on a previous occasion) was visible at the anterior end of the body. In many cases the females were enormously distended with spermatozoa, which could hardly have been all received at one time.

"The common occurrence of great numbers of spermatozoa in the body of the supposed female might suggest that *D. tæniatus* was hermaphrodite. Such a supposition is rendered sufficiently improbable by the following considerations: (I) That no other species of *Dinophilus* is known to be hermaphrodite; (II) that the process of fertilization was frequently observed in *D. tæniatus*; (III) that the spermatozoa so constantly seen in the female of the same species were, without exception, ripe and actively moving, no trace of spermmorulæ or unripe spermatozoa being discernible. Such stages in the development of the spermatozoa were never missed in any adult male individual."

4. *Chætopods.*

a. THE TUBIFICIDÆ. — Spermatophores are of very general occurrence among the oligochætous annelids. They were observed in the seminal vesicles of the Tubificidæ as long ago as 1828 by Dugés, and in 1850 by Budge; but their significance escaped these older authors, and even as late as 1861–62 Claparède described them as *opalina-like* parasites, under the name of *Pachydermon acuminatum* and *P. elongatum*. It was not until 1869, in a joint work with Metschnikoff, that Claparède recognized the real nature of his “opalinoid parasites.”

In 1848 Kölliker saw the spermatophore of *Spio* attached to the surface of the worm, and supposed it to be a *Gregarina*. The first to discover their meaning, according to Vejdovsky, was Doyère (1854–55).

In some of these spermatophores the tails of the inclosed spermatozoa project through the wall of the capsule, giving it the appearance of being clothed with vibratile cilia. Such spermatophores, in motion, resemble living organisms so closely as to deceive the trained eye, as the *Pachydermon* of Claparède well illustrates. Sometimes, as in *Psammoryctes*, these vibratile portions adhere in bundles on the neck of the capsule, giving it the appearance of being armed with recurved hooks like the proboscis of an *Echinorhynchus*. Vejdovsky himself first described these as “Widerhaken,” but corrected himself in his later monograph.

Among the earlier contributions to a more definite knowledge of these structures are two papers by E. Ray Lankester,¹ and one by Franz Vejdovsky.²

Lankester remarks on “The Structure and Origin of the Spermatophores of Two Species of *Tubifex*” (pp. 180, 181, 187) as follows:—

“The very curious structure of these built-up masses of spermatophores, the fact that they are an example of *a kind of organization* elsewhere without parallel, — a secondary aggregation, not due to growth as ordinarily presented by organized beings, but to accumulation of free independently developed elements, — gives them a claim on our attention, as well as the facts that they have been misunderstood by the

¹ Quart. Journ. Mic. Sci., 1870 and 1871.

² Zeitschr. f. w. Zool., XXVII, 1876.

ablest and latest writer (M. Claparède) on the animals which present them; and that they exhibit marked variations in form in the various genera and species of Oligochaet worms."

"Did we know of a number of free unicellular organisms after complete development becoming fixed together by a cement to form a secondary organism capable of locomotion and possibly of nutrition, we should have a parallel to the spermatophores; as it is, they are, I believe, the only examples of the building up of an organ or quasi-organism by agglomeration instead of histogenesis."

"The sperm-ropes of *Tubifex rivulorum* I have found in the copulatory pouches both in summer and winter, but especially abundant and well formed in the winter. They have a worm-like figure, with a curious conical head, an average from $\frac{1}{20}$ to $\frac{1}{15}$ of an inch in length, and from $\frac{1}{500}$ to $\frac{1}{200}$ of an inch in breadth, the narrowest part being that immediately succeeding the conical head, which has a breadth of about $\frac{3}{1000}$ of an inch."

"The general form of the sperm-rope is due to its being moulded in the long neck of the copulatory pouch. . . .

"It appears that the material of which the sperm-ropes are formed, namely, spermatozoa and a cementing matrix, must be introduced in a viscid form from the male efferent duct, through the penis of one worm into the copulatory reservoir of another, and in the neck of that reservoir a 'setting' occurs; for the sperm-ropes, when fully formed, are very firm and compact bodies, of high light-breaking power. The wall of the copulatory pouch is glandular, and undoubtedly furnishes a secretion which occupies part of its cavity, and in all probability also assists as a cementing material in the formation of the sperm-ropes."

b. THE LUMBRICIDÆ. — The account given by Vejdovsky¹ of the spermatophores of the Lumbricidæ comes more closely in several respects to what I have seen in the leeches. It is in these worms that spermatophores have often been seen attached to the surface of the body, usually on the first segments of the sexual girdle. According to Vejdovsky, they were known to the earlier naturalists of this century, and were usually described, after Morren's example, as "*appendiculæ generatrices*," or as "*penes*." Friedrich Müller (1849) first recognized them as spermatophores. Later (1857), however, they are referred to by Ewald Hering as "unimportant formations." Hering's description (Zeitschr. f. w. Zool., IV, 1857), nevertheless, seems to be of some value: —

¹ *System und Morphologie der Oligochäten*, Prag., 1884.

"Nach der Begattung tragen die Würmer meist in der Gegend des 26 Segments, *selten am Gürtel*, jederseits einen kleinen plattkolbenförmigen, ungefähr 1''' langen Anhang, den sogenannten Penis. . . . Er ist anfangs weich, wird aber allmählig härter und besteht aus einer hyalinen Substanz, in die am freien Ende ein Tröpfchen Samenmasse eingebettet ist. Er ist nachweisbar ein Product der Begattung und besteht nach meiner Ansicht aus *erhärtetem Schleime*."

Leuckart (Bericht, 1854-55) remarks as follows:—

"Referent hat an der vorderen Bauchseite der Regenwürmer zur Brunstzeit nicht selten kleine *spindelförmige Gebilde* angetroffen, die mit Samenfäden gefüllt waren und wohl als Spermatophoren zu betrachten sein dürften."

Fraisse (Semper's Arbeiten, V, 1879) devoted a special paper to the spermatophores of the earthworms, giving figures illustrating their form. Fraisse came to the conclusion that the outer homogeneous envelope of the capsule is not formed in the male efferent ducts, nor in the seminal vesicles, but probably by the glands associated with the sexual setæ. Vejdovsky finds the spermatophores attached sometimes in the intersegmental furrows, and considers this evidence against the glands ("tubercula") having any share in their formation. In opposition to Fraisse, Vejdovsky thinks it probable that the seminal vesicles furnish the secretion which forms the envelope in question.

Vejdovsky concludes his account with some remarks about the difficulty of understanding how the spermatozoa inclosed in spermatophores can reach the eggs. The female genital pore is located in the fourteenth somite, and the spermatophores are usually placed in the twenty-seventh to thirtieth somites. Furthermore, no external opening could be found in the spermatophore, by which the contents could escape. These facts naturally suggest to me the possibility of the spermatophores emptying themselves as they do in Clepsine. Such a possibility, however, is not mentioned by Vejdovsky, who suggests that the spermatozoa may be freed during the formation of the cocoon.

The mode of attachment of the spermatophores appears to be precisely the same as in Clepsine.

"*Ihre Basis wächst mit der Cuticula des Leibesschlauches zusammen und ist von der letzteren überhaupt nicht zu unterscheiden.*"

5. *The Capitellidæ.*

Among the polychætous annelids and the Capitellidæ there seems to be little that can be said to fall in the direct line of our inquiry. In very many cases the sexual products are set free in the water, and external fertilization takes place without the intervention of spermatophores. In *Polygordius*, according to Fraipont, the whole body undergoes a regressive metamorphosis at sexual maturity; and the sexual products, as the result, perhaps, of mechanical pressure upon the body-wall, weakened by almost complete atrophy, burst through, and escape into the water. The animal does not survive the evacuation of its sexual cells.

In one of the Capitellidæ (*Clistomastus lineatus*), according to Eisig, the whole abdominal region, in which the sexual cells are lodged, undergoes a histolytic metamorphosis at sexual maturity; and the sexual elements are set free by fragmentation of this region. The thoracic region alone remains intact, and this is supposed to be able to regenerate a new abdomen.

In Capitella we find spermatophores, but no evidence that they are ever attached to the exterior. Dr. Eisig has made a number of most interesting discoveries in regard to the mode of copulation and fertilization in these worms; and although they do not touch very closely the phenomena under consideration in the leeches, I think they are not so remote as to be out of place here.

Monog. XVI. Fauna und Flora von Neapel. 1887. pp. 284, 674-75, 790-93.

The organs of copulation in *Capitella* consist of (1) two pairs of modified hæmal parapodia belonging to the eighth and ninth somites; (2) a copulation gland lying between the genital parapodia of the ninth somite; and (3) a pair of genital sacs in the eighth segment, which function as *vesiculæ seminales*, *vasa efferentia*, and *penes*. The female possesses a like pair of genital sacs in the same segment, and these serve not only as organs of copulation (*vulvæ*), but also as *receptacula seminis* and *oviducts*. Dr. Eisig thinks the glandular *porophore* of the female, like the clitellum of the oligochæta, assists in copulation by its sticky secretion.

From the position of the copulatory organs, Dr. Eisig con-

cludes that in copulation the two individuals lie back to back, and that the terminal portions of the genital sacs of the male are everted and inserted as penes in the corresponding non-everted parts of the female.

The spermatozoa, originating like the ova in a so-called "genital plate," fall into the body-cavity in an imperfectly developed state; after attaining maturity, they collect in the genital sacs, and are there united into bundles or spermatophores [p. 793]. In this form they may pass, during the act of copulation, into the genital sacs of the female, thence into her body-cavity, where they meet and penetrate the ova. Fecundation is thus accomplished before oviposition.

Although Dr. Eisig does not seem to have witnessed the act of copulation, his statements rest on many concurrent evidences.

The most remarkable feature of this copulation remains to be mentioned. Dr. Eisig reports [pp. 791-92] that the ripe males copulate not only with adult females, but also with unripe females, and even with juvenes in which the sex has not yet become manifest; and what is still more astounding, *with young individuals of their own sex*. The proof of this lies in the fact that the genital sacs of young males were found stuffed with spermatophores before their genital plates had come into functional activity. They must therefore have received their spermatozoa from without.

These facts prove that the male is capable of discharging his function effectively without the aid of any co-operative act on the part of the female, and in this respect the copulation bears some resemblance to that of Clepsine. But there seems to be no evidence of spermatophores attached to the exterior.

6. *Arthropods.*

The spermatophores of *Eupagurus* were seen by Swammerdam (*Bibel der Nature*, p. 87), and described as "lauter regelmässigen Theilchen." Whether they represented eggs or spermatozoa was left undecided. About a century later, in 1841-42, the decapod spermatophores were rediscovered by Kölliker, and their purpose and origin made known through his observations and those of Von Siebold (*cf.* Mül. Arch., 1842, pp. cxxxv-vi). It seems probable, from Grobben's observations (Claus's *Arbeiten*, I, 1881, p. 67), that all decapods produce spermatophores. The

question as to how the spermatophores empty themselves, and whether fecundation is internal or external, has received different answers. Grobben (*l.c.*, p. 75) does not attempt to decide by what agent the spermatophore is made to burst and discharge its contents. In the Brachyura they are supposed to be dissolved in the *bursa copulatrix* of the female. Leuckart (*Zeugung.*, p. 900) accounts for the escape of the contents of the spermacase thus: "The walls gradually harden and compress the contents until the capsule bursts, or (as in the case of *Astacus* and insects) until it flows out of the open end." Paul Meyer (*Jen. Zeitschr.*, 1877, p. 204) thinks it is not the water that causes the spermatophore to burst (in *Galathea* and *Pagura*), and suggests that the secretion with which the female fastens the eggs to its legs may be the agent that accomplishes this.

It is in *Peripatus* and the Copepods that we find modes of impregnation more or less closely analogous to what takes place in the Rhynchobdellidæ.

ADAM SEDGWICK. The Development of *Peripatus Capensis*. *Quart. Jour. Micr. Sci.* N. S. XCIX. July, 1885. pp. 453-54.

a. PERIPATUS.—"The ovaries contain spermatozoa, *some of which project through the ovarian walls into the body-cavity*. This condition has been figured and described by Moseley.¹

"The ovaries always contain spermatozoa, but in smaller numbers directly after the eggs have passed into the oviduct than at any other time. This is a very marked feature of an ovary, say, at the beginning of April, when compared with an ovary from which the ova have just passed into the oviducts, say, at the beginning of May, the former being of an opaque white color to the naked eye, while the latter has a much more transparent appearance.

"This fact would seem to imply that fresh spermatozoa pass each year into the ovaries. This brings me to the question of the manner in which the male discharges his function. The *vesiculæ seminales* (testes of Moseley and Balfour) are almost empty of spermatozoa in the months of February, March, and April. At the end of April, however, they begin to swell again and contain spermatozoa, which increase in number as time goes on, until, in October, they are fully distended with spermatozoa in all stages of development. *There seems to be no functional intromittent organ, but the male deposits little oval spermatophores quite casually on any part of the body of the female, and, for all that I know,*

¹ Phil. Trans., Vol. 164.

of the male also; e.g. *I have often seen them on the head. How these little packets of spermatozoa get into the vagina, and then up the uteri, which are always full of embryos, I cannot conceive.* The spermatozoa exhibit a certain amount of vibratory movement, and no doubt, once within the vagina, they are set free from the spermatophore and make their way up the female generative tube, between the embryos and the uterine walls. Inasmuch as the deposition of spermatophores lasts from June until January, *each female probably has a large number of spermatophores deposited on her, and some of these are probably near the generative opening, and are, somehow or another, transported through it into the vagina.*

“Fertilization is apparently effected in the ovary. I have never seen spermatozoa in any part of the female apparatus except in the ovaries, and in small numbers in the upper end of the oviducts at the time when the ova are entering the latter.”

AUGUST GRUBER. Beiträge zur Kenntniss der Generationsorgane der freilebenden Copepoden. *Zeitschr. f. w. Zoology.* 1879. p. 407.

b. COPEPODA. — Fertilization is accomplished by means of spermatophores in the Copepoda; but here the sperm-cases are always, so far as known, affixed to the body of the female in the immediate neighborhood of the genital openings, and the contents are never forced through the body-wall.

The whole history of the spermatophore has been carefully traced out by Gruber. Its formation in *Hetercope* is considered to be typical for the entire group.

The *vas deferens* forms two secretions, one of which forms the elongated, sausage-shaped spermatophore, while the other is incased along with the spermatozoa. The spermatophore is attached to the vulva of the female by one end, which is drawn out into a tubular neck. As soon as this is accomplished, the contents of the capsule begin to flow out, as the result of a sort of histolytic metamorphosis which overtakes the larger part of the inclosed spermatozoa. These swell up to pale spherules, which grow larger and larger, fuse, and finally give rise to a network of polygonal spaces. Meanwhile the inclosed secretion has been gradually expelled, and now forms a slimy mass in the vulva, into which, as a sort of secondary capsule, the remnant of spermatozoa is driven. Oviposition follows. The eggs on their way out of course meet the spermatozoa, and both are carried out together.

In those species of the Calanidæ which have one or a pair of *receptacula seminis*, the phenomena are the same, except that the spermatophores are attached to the pores of these receptacles, and their contents pass into the receptacles, instead of directly into the *vulva*. The spermatozoa find their way into the vulva through connecting ducts, when the eggs appear.

In the Calanidæ, the male catches the spermatophore, as it is extruded, with his fifth pair of limbs, and then places it on the body of the female. The secretion which begins at once to escape from the neck of the capsule serves to fix the latter first to the limb and then to the genital pore of the female [p. 424].

In other families, the male and female lie with their ventral surfaces together; the male seizes with his antennæ the last pair of swimming-legs of the female, then bends his abdomen forward, and fixes the spermatophore directly on the opening of the *receptaculum seminis* by means of a special secretion [pp. 424-25].

c. GAMMARUS. — Della Valle¹ states that the oviducts end blindly, and that what should be the opening at the base of the fifth pair of legs is completely closed, except at the moment of oviposition.

Copulation occurs while the female is still bearing young in her pouch. The young leave the pouch; and the female moults, the male assisting in the operation. The ventral surfaces are together, so that the papillæ of the male are in apposition with the region of the oviducal extremities of the female. As soon as the moulting is finished, ejaculation of spermatozoa occurs: the ova appear half an hour later. The spermatozoa are not inclosed in sperm-cases, but they adhere to the ventral surface of the fifth segment and on the plates of the pouch. The oviducts are forced open by pressure from within, and the ova are covered with a viscid gelatinous mass which binds them and the spermatozoa together. For this abstract I am indebted to Dr. McMurrich.

C. SPENCE BATE. Report on the Present State of our Knowledge of the Crustacea. *Report British Assoc. f. Adv. of Sc.* 1880. pp. 230-32.

d. ASTACUS. — "Copulation of the crayfish takes place, according to the observations of M. Chantran,² during a period which includes the

¹ Atti. Soc. Nat. Modena (3), VIII, 1889.

² Comptes Rendus, July 4, 1870, LXXI, pp. 42-45; Ann. Nat. Hist., 4th Ser., Vol. VI, p. 265.

months of November, December, and January. The male seizes the female with his large nippers, turns her over, and whilst he holds her lying on her back, places himself in such a manner as to pour out the fecundating material upon the two outer lamellæ of the tail. After this first operation, which lasts some minutes, he conveys her rapidly beneath his pleon, in order to effect a second deposition of semen upon the plastron round the external opening of the oviducts, by means of the curious mechanism so accurately described by M. Coste, upon the plates of the caudal fan. (*Ripisura*.)

“According to the degree of the maturity of the ova at the time of the union of the sexes, oviposition takes place at a period varying from ten to forty-five days after copulation. . . . Immediately after oviposition (which usually takes place during the night, and is accompanied with an emission of mucus for securing the eggs) we may detect in this mucus and water the presence of spermatozoids, precisely similar to those which are contained in the spermatophores attached to the plastron, and derived from them.”

These spermatophores still remain attached to the plastron long after oviposition.

“They consist of small white coriaceous filaments, either isolated or mutually adherent; they no longer show anything but a central cavity, in which the microscope reveals only a few more or less withered spermatozoids. The wall of these spermatophores retains its thickness, and remains, as before, composed of a concrete, striated, tenacious mucus.”¹

Fecundation [p. 230] is thus accomplished after oviposition. This is concluded from the fact that spermatozoa are found in the mucus surrounding the eggs, and from the fact that the spermatophores are then empty.²

POSTSCRIPT.

Dr. C. T. Hudson, who has recently given us the results of observations continued for upwards of thirty years on the Rotifera, seems to doubt Plate's statements regarding the injection of spermatozoa through the body-wall. In his presidential

¹ Comptes Rendus, Jan. 15, 1872, LXXIV, pp. 201-2; Ann. Nat. Hist., 4th Ser., IX, pp. 173-74.

² Cf. A. Lereboullet: *Recherches d'Embryologie comparée sur le Développement du Brochet, de la Perche et de l'Ecrevise*. Paris, 1862. p. 652.

address¹ to the Royal Microscopical Society, referring to Plate's account, he says: "It is not necessary to comment further on this strange theory than to say that Gosse has seen intercourse take place at the cloaca in the case of *Brachionus pala*; M. E. F. Weber, in that of *Diglena catellina*; and Mr. J. Hood, not only in *Floscularia ornata*, *Synchaeta gyrina*, *Euchlanis triquetra*, and *Melicerta tubicolaria*, but also more than a score of times in *Hydatina senta* itself."

"The frequent presence of spermatozoa in the perivisceral cavity" is, however, one of "the doubtful points" for which Dr. Hudson offers no explanation. No opening is known by which they could reach this cavity. "Neither the oviduct, nor the cloaca, is known to have an opening into the perivisceral cavity, and yet the spermatozoa in several species have been seen in that cavity, *adhering to the outside of the ovary*. How did they get there?"

Plate's observations answer this question, and it will not do to dismiss them as "a strange theory," since the same strange thing is reported from so many different sources.

¹ *On Some Doubtful Points in the Natural History of the Rotifera*. Journal Royal Microscopical Society, February, 1891, p. 6.

EXPLANATION OF PLATE XIV.

LETTERS.

I-XXXIII = somites.	<i>nc.</i> = ventral nerve-cord.
I-66. = number of rings.	<i>n.</i> = nerve.
<i>al.</i> = alimentary canal.	<i>nph.</i> = nephridial pores.
<i>alc.</i> = gastric cæcum.	<i>o.</i> = outer layer of the spermatophore.
<i>b.</i> = brain.	<i>æ.</i> = œsophagus.
<i>c.</i> = cœlomic cavity leading up to the ovaries.	<i>ag.</i> = œsophageal pair of glands.
<i>ca.</i> = cæcal appendage of the ovary.	<i>ov.</i> = ovary.
<i>c.ep.</i> = cœlomic epithelium.	<i>p.</i> = proboscis.
<i>cg.</i> = caudal appendage.	<i>phg¹.</i> = first pair of pharyngeal glands.
<i>d.</i> = ductus ejaculatorius.	<i>phg².</i> = second pair of pharyngeal glands.
<i>es.</i> = egg-string.	<i>s.</i> = enlarged end-portion of vas deferens commune.
<i>f.</i> = fibrous prolongation.	<i>sp.</i> = spermatozoa.
<i>g.</i> = glandular part of the ejaculatory duct.	<i>t.</i> = testes.
<i>gs.</i> = granular secretion.	<i>vd.</i> = vas deferens.
<i>i.</i> = inner layer of the spermatophore.	<i>vd.c.</i> = vas deferens commune.
<i>lm.</i> = longitudinal muscles.	<i>vs.</i> = vesicula seminalis.
<i>lu.</i> = lumen of the base of the spermatophore.	<i>w.</i> = end of ejaculatory duct.
	<i>x.</i> = position of the spermatophore.
	<i>y.</i> = course of the spermatozoa.

FIG. 1. — Transverse section of *Clepsine plana*, showing cœlomic cavities filled with spermatozoa (red), which penetrated the epidermis at the level of the point marked X. The cœlomic cavities communicate with the cavity in which the ovaries (*ov*) lie at the point *c*. Only about one-half of the section is represented in the figure. X 25.

FIG. 2. — A part of one of the following sections, showing the base of the spermatophore and the spermatozoa issuing from it. The arrows show the direction of penetration through the muscular layers. X 120.

FIG. 3. — Section of cœlomic cavities with their peculiar epithelium. X 120.

FIG. 4*a*. — Spermatophore, just placed (3.4 mm. long), and full of spermatozoa. The granular substance issuing from the mouth seems to serve the purpose of preparing the way for the penetration of the spermatic elements. X 55.

b = section of the case two days after the escape of the spermatozoa; *c* = corpuscles filling the mouth of the spermatophore. X 280.

FIG. 5. — The central nervous system and sexual organs, together with the proboscis, œsophagus, and the so-called salivary glands. X 4½.

FIG. 6. — The supra and sub-œsophageal ganglia, seen from the dorsal side. X 50.

FIG. 7. — The same, seen from the side. X 50.



DESCRIPTION OF *CLEPSINE PLANA*.

C. O. WHITMAN

THIS species, as already stated in the foregoing paper on Hypodermic Impregnation, agrees in some features closely with *C. parasitica*, as described by Say¹ and Verrill²; but the points of agreement are not sufficient for identification; for I have four quite distinct species, — and I have reason to think there are at least several more, — each of which comes about equally near the characters said to belong to *C. parasitica*. In view of the fact that we have several large species of Clepsine which agree in having a single pair of eyes, a median yellow vitta, and marginal yellow spots, I am led to doubt the identity of Verrill's *C. parasitica* with that described by Say. Say says, "This leech is frequently found in the lakes of the Northwestern region, adhering to the sternum, or inferior shell, of tortoises (*Emys*), particularly to that of *E. geographica* of Lesueur." Verrill's specimens were found in "West River, near New Haven, Conn., on the lower side of floating wood, and at Norway, Me." I have found two quite distinct species of these large Clepsines in the vicinity of Milwaukee, either of which may, or may not, be Say's species. I have two eastern species, quite distinct from each other and from the two Wisconsin species; one from Charles River, Watertown, the other from a pond in Worcester. Whether one, or the other, or neither of these is identical with Verrill's species, I am unable to say.

Say mentions as the constant characters: "A yellow vitta before; *quadrate* marginal spots each side; beneath with about eleven longitudinal lines; ocular points two." Then comes the following description: "Body dilated when at rest, narrowed before; above varied with dull yellowish and blackish brown;

¹ *Major Long's Expedition to the Source of St. Peter's River, etc., in 1823.* Keating's compilation, Vol. II, Appendix, p. 14. London, 1825.

² *Synopsis of the North American Fresh-Water Leeches.* Professor Baird's Report for 1872-73, p. 678. The same description was given in the *American Jour. Sc. and Arts*, Vol. III, February, 1872, p. 128.

a yellow vitta commences at the anterior extremity, and is more or less elongated, in some specimens less than one-fourth the length of the body, and in others extending nearly, or quite, to the posterior disc; lateral margin with eighteen or twenty symmetrical, equal, and equidistant quadrate yellowish spots; posterior disc above radiate with yellowish; ocular points two, appropriate, sometimes apparently confluent; beneath very flat, whitish, with about eleven longitudinal lines; lateral edges very acute.

"Length, in a state of repose, two inches; greatest breadth, seven-tenths of an inch."

According to Verrill: "This species is one of the largest and most conspicuously colored of the genus.

"Body smooth, but distinctly annulated, much depressed, broad, tapering anteriorly to the obtusely rounded head, broad and emarginate posteriorly, with a broad, round, posterior sucker or acetabulum, about half of which is exposed behind the end of the body. Length, in extension, three inches; greatest breadth, three to five tenths of an inch, according to the degree of extension. Ocelli usually united into one inconspicuous spot, placed near the anterior margin of the head; two or three other minute black spots, somewhat resembling ocelli, sometimes occur along the margins of the head anteriorly.

"Upper surface variegated with green, yellow, and brown; the ground-color is usually dark greenish brown, with a broad median vitta of pale greenish yellow, which at intervals expands into several large, irregular spots; unequal, oval, and rounded spots are also irregularly scattered over the back. The entire margin is surrounded by a series of alternating square spots of dark green and yellow. Lower surface longitudinally striped with numerous purplish brown and black lines; the margin spotted like that of the upper side."

These descriptions give us the size, shape, color, number of eyes, and locality. Size and shape do not help us much, since they vary so much, and since so many species are so nearly alike in these respects. The eyes are a very constant feature; but a number of distinct species agree in having only one pair of eyes closely approximated near the anterior margin of the head. The localities are so widely separated that they speak against rather than for identity. Color, as every one knows

who has had any experience in collecting and describing leeches, is a very unsatisfactory guide to identification. We do not find then in these descriptions either a single character or a combination of characters that may not belong to any one of several different species.

Perhaps the claim might be made that "all these species are apt to be quite variable in character in different localities, as well as at different periods of growth" (Verrill, *l.c.*, p. 677). This is undoubtedly true of most of the characters above named. But is it true that the variability is so great and so general that distinctive characters are nowhere to be found, either in the external or the internal organization? In other words, do the "species" and "varieties" grade into one another so closely as to make it impossible to find really *distinctive* characters? Must we be content with descriptions that only help us to bring together more or less nearly related forms, and give up the attempt to find constant morphological features which may serve as a reliable means of identification? It must be admitted that most authorities—if we may judge from their descriptions—have taken this view. The result is that we still have no uniform method of describing the Clepsinidæ, and very few descriptions that are not more a hindrance than an aid to progress in their classification. This is a simple statement of fact, and not a criticism reflecting upon any particular author's work. Early classifiers of the Hirudinea adopted what now appears to be an altogether inadequate mode of description, but which was perhaps all that the times seemed to require. The example first set by European systematists has naturally enough been followed by later authorities, in this country as well as in others, and no one can be justly reproached for not having foreseen the necessity of a better system. If, then, I attempt to point out defects of method, and to suggest how they may be improved, I trust no one will find cause for thinking that I am actuated by a spirit of captious criticism, or with any desire to belittle the labors of others. The defects to be pointed out are not the defects of any particular piece of work alone, but rather those of a long-standing and generally received method of describing the Hirudinea. The above descriptions of *Clepsine parasitica* are neither the best nor the worst of their kind; but they are fairly representative, and are here made the sub-

ject of special remark merely because they happen to touch the case in hand.

Let us now consider briefly in what particulars these descriptions fail to meet our needs. First of all they are not accompanied with any figures, and without such aids it is often extremely difficult, if not impossible, to get at the author's exact meaning, however skilful he may be at depicting. The oral sucker in these species presents a number of important diagnostic characters, not one of which is mentioned. The position of the mouth varies for different species, but it is not alluded to. Important specific distinctions are to be found in the number and condition of the rings represented in the head, and especially in the buccal and post-buccal rings, all of which are ignored. Nothing whatever is said about the number of rings and segments in the animal, and the peculiarities of the abbreviated posterior somites pass unnoticed. The metameric sense-organs were not then known, and although conspicuous enough, escaped notice. The nephridiopores are neither numbered nor otherwise defined, and the genital pores are not so much as mentioned. All this for the external features. Not one of the internal features received any notice, not even the diverticula of the stomach.

Some time ago I attempted to show that a satisfactory basis for the classification of the ten-eyed leeches (*Hirudinidæ*) was to be found in the external metamerism. I shall now show that the same method may be extended to the *Clepsinidæ*. The determination of the number and homology of the rings is often more difficult here than in the *Hirudinidæ*; but still it is always possible, I believe, and it affords the only safe basis for distinguishing genera and species. It is the remarkable constancy of these metameric characters that gives them such high value for diagnostic purposes. With metamerism as a basis, all the external features are readily defined topographically, and with a precision and definiteness that cannot otherwise be attained. The first thing to be done in describing a new form is, therefore, to determine with all possible precision the number of the rings and somites. This task is comparatively easy except at the two extremities of the body, where, it must be confessed, it is often quite difficult to decide upon the number of rings. This is more especially true of the head region. Here it is often

necessary to resort to various expedients. It is well to have several individuals killed in weak chromic acid ($\frac{1}{8}$ to $\frac{1}{4}\%$), in an extended condition. If the leeches do not die extended, they may easily be straightened by stretching a little. After lying in the acid a short time, the rings usually become sufficiently well defined to admit of study and comparison. It is advisable always to supplement this study with that of the living leech. Sometimes I have found it necessary to cut off the head and study it in all positions while it is contracting and expanding. For such study, a good dissecting microscope (*e.g.* that of Zeiss) is indispensable. The metameric sense-organs enable one to fix the limits of the somites, and are thus a most important guide in the analysis of the head region. Having determined the composition of the first two or three somites, the next important point to settle is the number of the ring which forms the posterior boundary of the oral sucker. This buccal ring is sometimes united either above or below with the preceding or following ring, and hence both the buccal and the post-buccals require careful examination from all sides and in all states of contraction and extension. The ocular ring is frequently a double ring, *i.e.* two rings more or less consolidated. In order to describe accurately such rings, it is necessary to note the relative width of the successive rings, particularly in the head and the posterior extremity of the body. The method of numbering rings and somites is shown in Figs. 1 and 3, Pl. XV.

My chief reason for offering the following description at this time is the fact that I have had to refer to this leech by name in my paper on spermatophores, and it seems desirable to show that the name stands for a reality. The description may, however, be regarded as a preliminary one, inasmuch as I hope to be able to describe this in connection with the more closely allied species, and to furnish with the descriptions the much-needed colored figures. The preparation of such figures, with due attention to all the details which require to be accurately reproduced, is already in progress.

CLEPSINE PLANA, *n. sp.*

The largest of the five specimens obtained measured as follows:—

Length at rest, 5-6 cm. ; width, 2.6 cm.

Length in extension, 8.5 cm. ; width, 1.8 cm.

Head, 4 mm. wide, scarcely marked off from the body, obtusely pointed in extension, rounded or truncated at rest.

Body, ovate-elliptical in contraction, emarginate posteriorly, very thin, showing two rows of very low, smoothly rounded, metameric protuberances on the dorsal surface, and between these similar, but scattered, non-metameric protuberances.

Disc, 9 mm. in diameter, circular, often largely covered by the body.

Annuli, 66 between the eyes and the anus, counting four double rings (1, 2, 64, 65) as single rings.

Somites, XXVI in front of the anus, XXXIII in all.

Buccal annuli = 5th and 6th, united for about the middle third of the ventral side, distinct towards the margins of this side.

Post-buccals = 7th and 8th, completely united below.

Eyes, 2, rather obscure, and in contact at the anterior edge of the first ring. In the young the eyes are conspicuous and quite distinct, although nearly or quite contiguous.

Mouth, in front of the centre of the flat oral sucker.

Genital pores. — Male orifice in the 10th somite, between 24th and 25th rings ; female orifice in the 11th somite, between 26th and 27th rings.

Testes = six pairs in 12th to 17th somites.

Anus, behind the 66th ring, between this and a postanal rudiment, representing probably a remnant of one or two rings.

The annuli of the head. — In front of the eyes I was unable to discover any distinct rings. In another species, *C. chelydræ*, from Wisconsin, there are three narrow rings in front of the eyes ; and the first is marked by the usual metameric sense-organs. Although no metameric sense-organs were recognized in front of the eyes in *C. plana*, the correspondence of other metameric characters in the two species is sufficiently close to enable me to identify the ocular rings as equivalents. The pre-ocular part of the head is, therefore, probably equivalent to the first somite of *C. chelydræ*, and is so numbered in Fig. 1. The ocular ring is double, representing the 1st and 2d rings of the second somite, so incompletely divided that the evidence of duplicity is seen mainly in the relative width of the ring.

This ring, then, corresponds to the 4th and 5th rings of *C. chelydræ*.

The 2d ring (6th and 7th of *C. chelydræ*) is also double, as shown by its width and by a slight division, sometimes noticeable at the margins. This ring represents the third ring of the second somite, united with the first ring of the third somite, as is plainly shown by the sense-organs being placed in the posterior half of the ring.

The 3d and 4th rings are of equal width, and slightly narrower than the double rings preceding them. These, together with the posterior and sensory half of the 2d ring, constitute the third somite.

The 5th (sensory) and 6th rings (buccals) are a trifle wider, distinct above, but united below except at the margins. These two rings form the posterior limit of the head, and together form the first ring behind the suctorial surface of the ventral side. When the leech is at rest, with the head attached, a feeble constriction may usually be seen, which falls between the 6th and 7th rings, and thus obscurely marks off the head.

The 7th and 8th rings, the post-buccals, are distinct above, but consolidated below. From this point onward the rings are regular and distinct, both above and below, until we reach the twenty-third somite.

In the head we have found only two incomplete somites (1st and 2d), *i.e.* somites with less than three distinct rings. The twenty somites, from III to XXII inclusive, are complete in respect to the number of constituent rings. The body terminates behind with four short and incomplete somites (XXIII-XXVI). The twenty-third has two distinct rings (62d and 63d); the twenty-fourth, one plainly double ring (64th), consisting of a sensory ring and a narrow, imperfectly defined rudiment; the twenty-fifth, one ring (65th), giving evidence of its double nature only at the margins; and the twentieth-sixth, one narrow and simple sensory ring (66th). Adding seven somites for the disc to the twenty-six pre-anal somites, we have as the whole number thirty-three, which agrees with the number of ganglia in the ventral chain.

In regard to the abbreviated somites, it will be noticed that we have here, as in all the other Hirudinea, the greatest reduction in the number of rings in the two end somites. Reduction,

as I have before pointed out, seems to have begun at both extremities, and to have advanced from these points towards the middle region of the body. Its advance has been centripetal, and the extent of its advance shows how far a form has departed from the ancestral condition of uniform somites. It is here that we discover a very important guide to the systematic rank and relationship of different forms. This is most clearly illustrated in *Hirudo* and *Hæmadipsa*.

It may be well here to call attention to a fact hitherto overlooked; namely, that metamerism among the leeches has undergone modification in two opposite directions. Variation by centripetal *reduction* of the number of rings is universal; variation by *multiplication* of rings characterizes, as a rule, only the higher forms, *Hirudo*, *Nephelis*, etc. *Clepsine* rarely exhibits the second mode of variation, and never to the extent that *Hirudo* does. The difference between the *Clepsinidæ* and the *Hirudinidæ* in this respect has a physiological explanation. *Hirudo* swims, and for this purpose a long flexible body is required; *Clepsine*, with few exceptions, habitually creeps, and for this mode of locomotion, supplementary rings have not been essential. In variation by multiplication we have another means of determining close systematic relations.¹ I would not be

¹ I am reminded of an error into which I fell in my paper on Japanese Leeches. The error was the assumption that all somites having less than five rings were abbreviated. The assumption should have been, as I now feel convinced, that all somites with less than *three* rings are abbreviated, and all with more than three have been increased by the division of one or two of the three primary rings. I have collected considerable evidence, which cannot be given here, going to show that in the evolution of *Hirudo*, it was the second and third rings that underwent division, while the first remained undivided. In the *Hirudinidæ*, then, we have *supplemented somites* (five rings, rarely four), *type-somites* (three rings), and *abbreviated somites* (0-2 rings). The type-somites I formerly regarded as abbreviated. The view here taken helps to understand what before seemed unaccountable, that *Hirudo* and most of its congeners present *three successive somites* (4-6) with only *three* rings each. Allowing these to be type-somites, we recognize in them a sort of *neutral zone*, standing between the abbreviated and the supplemented somites. Usually one of these type-somites only is preserved in the posterior region of the body; and sometimes we find this somite already enlarged to *four* rings, by the division of its *third* ring, as is well shown in the Japanese *Leptosoma acranulatum*.

Mr. Apathy—who, as I observe, seems to look upon “Some New Facts about Leeches,” which I recently published, as worthy of being claimed as his own discoveries—advances a different view, according to which the type-somite is supposed to have had twelve rings. A review of his position must be postponed until I can bring forward the evidences which seem to me fatal to such a view.

understood as claiming that variation in these ways can always be relied upon as an exact gauge of systematic relationship. I am not unmindful that in such questions the *entire* organization must be the final criterion in doubtful cases. Nevertheless, I hold that we find in the variations above defined an important guide.

Color. — Ground-color above a dull, dark brown, with a slightly darker marginal border (Figs. 1 and 3) encircling body and disc. Along the margin there are twenty-one dull yellow spots, metamerically arranged, and having the same width as the dark border. The first spot is very small and on the ocular ring, the second on the second ring, the third on the two buccal rings, the fourth mainly on the ninth and tenth rings. From this point onward the spots mark the second and third ring of each somite, except the last four (XXIII–XXVI), in which they are absent. Anteriorly these spots have a triangular form with the apex directed towards the median line. In the sixth somite they begin to assume a V-shaped form, and this passes into a U-shaped form along the middle and posterior regions.

The median portion of the tip of the head is whitish up to the eyes. With the eyes begins the median yellow vitta, constricted at irregular intervals. This vitta runs back to the fourteenth ring, and then fades into an obscure patch of brownish yellow reaching back to the twentieth ring. The extent of the vitta is quite variable, as was pointed out by Say and Verrill.

There are two rows of yellow spots metamerically arranged, marking the first ring of each somite (from VII onward), and situated about midway between the margin and the median line. These spots are at first small, circular, and placed just inside the lateral row of sense-organs (*l*). From the twenty-sixth ring backward, they become slightly elongated so as to reach and encircle the corresponding sense-organs. All the sense-organs of this row, anterior to the twenty-sixth ring, are encircled with a narrow border of yellow, except the first two pairs (on 2d and 5th rings). The two median rows of sense-organs are not thus marked, until we come to the last three pairs, which have quite conspicuous borders. The outer lateral sense-organs (*ol*) are so marked only as far as the seventeenth ring.

Between the two rows of yellow spots there are other yellow spots, scattered irregularly along the dorsal surface, varying in

size from that of the larger metameric spots to much smaller dimensions. All these spots, the metameric as well as the non-metameric, mark low, rounded protuberances. The lateral sense-organs (*l*) are not placed at the summit of these protuberances, but close to the base, on the outer side.

On the disc we find yellow patches arranged in about six radial lines, in each of which are seen from two to three sense-organs. These radial rows of sense-organs correspond to the median (*m*), lateral (*l*), and outer lateral (*ol*) rows of the body.

The ventral surface is paler; the marginal dark border and yellow v- or u-shaped spots are the same as above, only paler. There are thirteen light longitudinal streaks alternating with dark streaks (twelve in number) (Fig. 2).

On the ventral side I find six rows of sense-organs, differing from those of the dorsal side only in being smaller and more difficult to find. In all, then, we have fourteen rows of these sense-organs, as in *Hirudo*.

The *nephridial pores* (*nph.*) lie in the first ring of the somite, just in front of the line dividing the ring into an anterior and a posterior half. They are ranged along the medial edge of a broad, light streak, on the outer edge of which are seen the lateral sense-organs.

The *stomach* has seven pairs of much-branched diverticula; the seventh diverticulum has five lateral cæca.

The *sexual organs* have been figured and described in connection with the subject of spermatophores (Pl. XIV, Fig. 5).

In the same place I have figured the entire central nerve-system, and given two enlarged views of the supra- and infra-oesophageal ganglia (Figs. 6 and 7).

The number of segments represented in the infra-oesophageal ganglia is five, as shown by the number of nerves, and by the presence of ten median ventral ganglia (1-5, Fig. 7). The arrangement of these ventral ganglia, shown in the accompanying figure, may be of some value in identifying the species.



FIG. 1. — Ventral ganglia of the infra-pharyngeal portion of the central nervous system, showing that five metameres are represented in this region.

EXPLANATION OF PLATE XV.

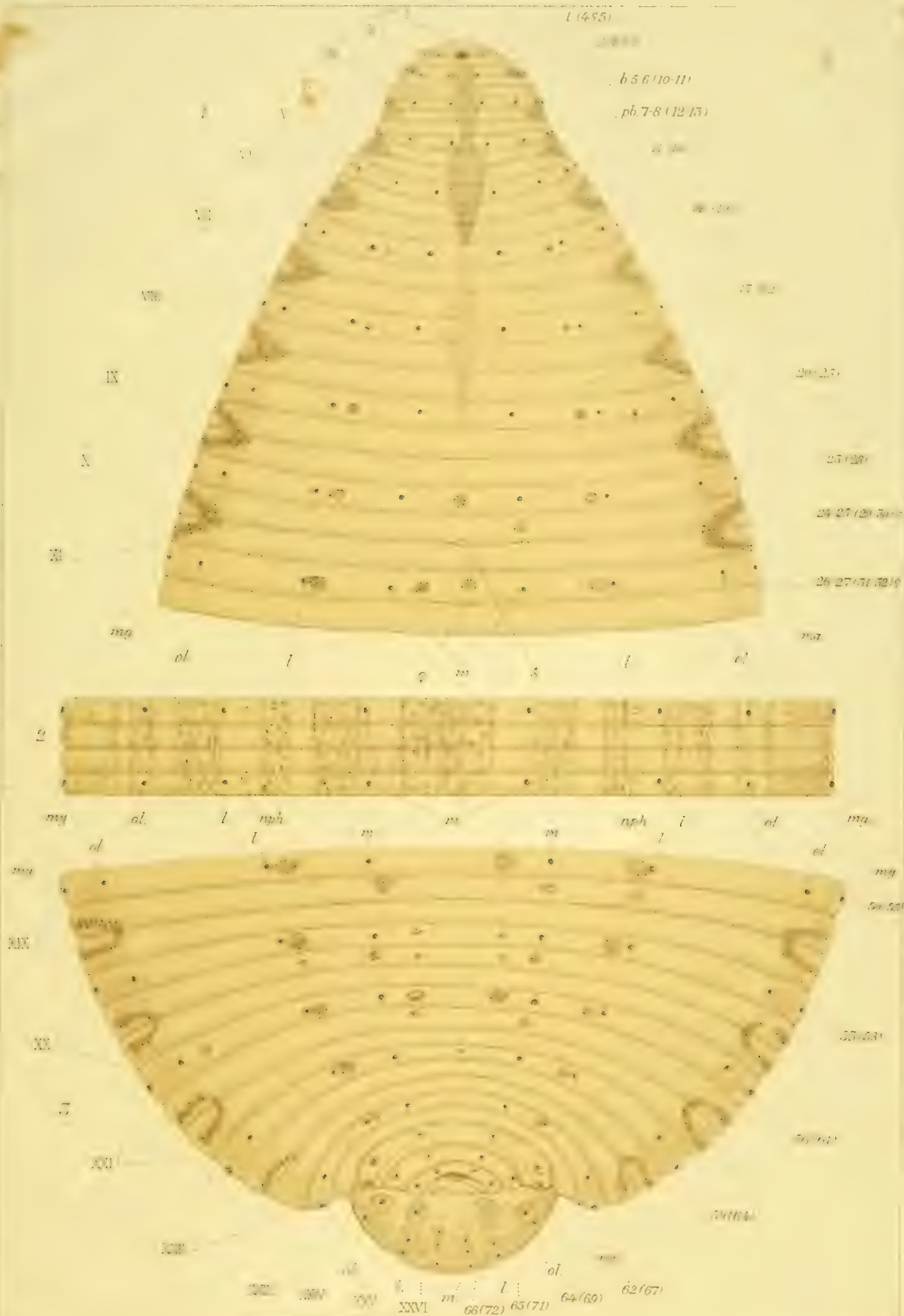
LETTERS.

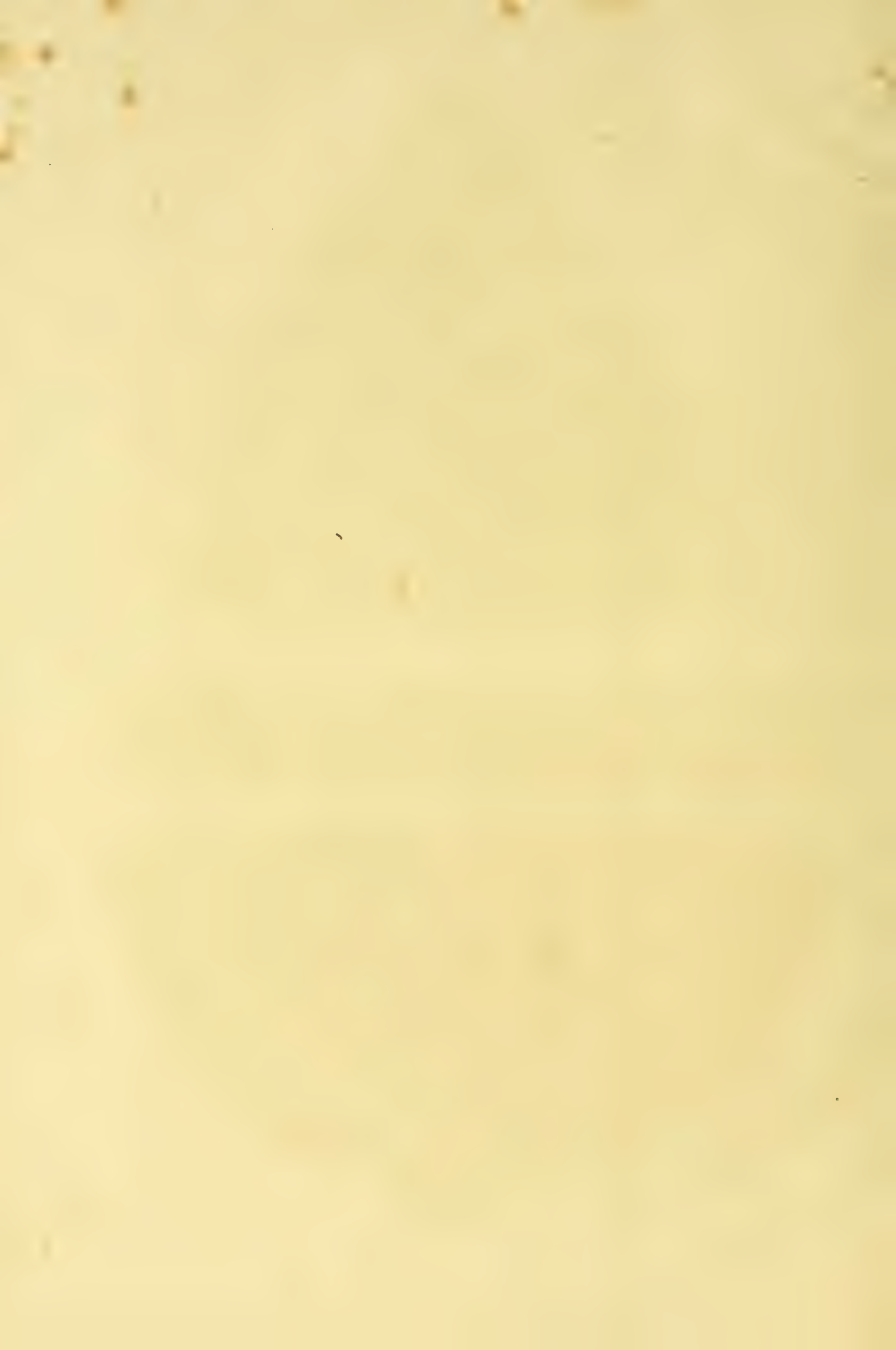
I-XXVI	= somites.
I-66	= rings.
(4-72)	= corresponding rings of <i>C. chelydræ</i> .
b. 5-6	= buccal rings.
pb. 7-8	= post-buccal rings.
l.	= lateral row of sense-organs.
m.	= median row of sense-organs.
mg.	= marginal row of sense-organs.
nph.	= nephridiopores.
ol.	= outer lateral row of sense-organs.

FIG. 1.—Diagram of the anterior portion of *Clepsine plana*. The first ring bearing the single pair of eyes is double, and corresponds to the fourth and fifth rings in *C. chelydræ*, which appears to be the more typical form. The second ring is also double, and equivalent to the sixth and seventh of *C. chelydræ*. The numbers inclosed in parentheses in this and the two following figures refer to the same species. Dotted areas show distribution of the yellow pigment. × 5.

FIG. 2.—Ventral surface of four rings from the middle of the body, showing the longitudinal pigment-lines and the relative position of the sense-organs, as well as the nephridial pores. × 5.

FIG. 3.—Diagram of the posterior portion of the leech, prepared with especial reference to the topographical relations of the sense-organs and the annular composition of the last four somites of the body. × 5.





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